ORAL HEALTH AND COGNITIVE FUNCTION IN THE ATHEROSCLEROSIS RISK IN COMMUNITIES (ARIC) STUDY

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ABSTRACT

SUPAWADEE NAORUNGROJ: Oral health and cognitive function in the Atherosclerosis Risk in Communities (ARIC) study
(Under the direction of Gary D. Slade and Victor J. Schoenbach)

Emerging evidence suggests possible links between poor oral health and cognitive dysfunction in older adults; however, it is unclear whether the association is present in midlife, and whether poor oral health in midlife increases risk of subsequent cognitive decline. The present study aimed to investigate whether a) periodontal disease and tooth loss were associated with low cognitive performance and b) periodontal disease and tooth loss were predictive of eight-year cognitive decline. The study used data collected from adults aged 52-75 years who participated in the dental health component of the Atherosclerosis Risk in Communities (ARIC) study.

Cognitive function tests consisted of Delayed Word Recall (DWR), Digit Symbol Substitution (DSS), and Word Fluency (WF). At ARIC Visit 4 (1996-1998), 9,874 participants also answered dental screening questions, and 5,942 of the 8,554 dentate participants received comprehensive oral examinations, including periodontal probing. From 2004-2006, cognitive function for 911 participants was reassessed as part of the Brain MRI study; 785 of these participants were dentate, and 558 of them had received periodontal examinations at ARIC Visit 4. Models fit with multiple linear regression and generalized estimating equations (GEE) used dental status, number of teeth, or clinical periodontal conditions classified by the Biofilm-Gingival Interface (BGI) classification to predict Visit 4 cognitive scores and eight-year change in cognitive scores, respectively. **In our cross-sectional study**, complete tooth loss was consistently associated with

lower performance on all three measures of cognitive function. Number of teeth and periodontal disease were associated only with DSS and WF scores. **In our cohort study**, mean scores from all three cognitive measures slightly decreased. Although we found that complete tooth loss was associated with low performance on two cognitive tests, the DWR and WF, our data did not support the hypothesis that poor oral health predicted greater cognitive decline. In these late-middle aged adults, complete tooth loss was significantly associated with low cognitive performance but not with subsequent cognitive decline. Although differences in mean scores between BGI groups were small, greater extent of gingival inflammation tended to correlate with lower cognitive scores.

DEDICATION

To my Advisors

Dr. Victor J. Schoenbach and Dr. Gary D. Slade

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TABLE OF CONTENTS

LIST OF TABLES x

LIST OF FIGURES xiii

LIST OF ABBREVIATIONS xvi

Chapter

1. LITERATURE REVIEW 1

A. Introduction 1

B. Dementia and cognitive decline 1

C. Determinants of cognitive decline, dementia, and structural brain abnormalities 3

D. Associations of oral health measures with cognitive function 7

E. Cognitive assessments 12

F. Neuroimaging 14

G. Periodontal disease assessments 16

H. Tables 19

I. Figures 20

References 21

2. SPECIFIC AIMS 28

A. Rationale 28

B. Specific aims 29

References 33

3. RESEARCH METHODS 36

A. Study design 36

B. Source of population 36

C. Data sources and participants' involvement 36

D. Sample size and power 38

E. Assessment of exposures, outcomes, and covariates 38

F. Statistical analyses 42

G. Protection of human subjects 47

H. Tables 49

I. Figures 51

References 52

4. STUDY 1: RESULTS 53

A. Overview 53

B. Introduction 54

C. Methods 56

D. Results 58

E. Discussion 60

F. Conclusion 63

G. Human participants protection 64

H. Tables 65

I. Figures 68

J. Supplemental materials 70

K. Supplemental tables 71

L. Supplemental figures 81

References 83

5. STUDY 2: RESULTS 85

A. Overview 85
B. Introduction....86
C. Methods 87
D. Result....91
E. Discussion 93
F. Conclusion....97
G. Human participants protection....97
H. Tables 98
I. Figures....101
J. Supplemental materials....104
K. Supplemental tables....106
L. Supplemental figures 119
References....123
6. DISCUSSION AND CONCLUSIONS 126
A. Summary of major findings....126
B. Strengths 129
C. Limitations....131
D. Public health significance....132
E. Future research direction....133
References....135
Appendix A: Summary of major findings....136
Appendix B: Directed acyclic graphs (DAGs)....143
Appendix C: Oral health measures....145
Appendix D: Cognitive function assessment in ARIC study 154
Appendix E: Characteristics of ARIC study participants....163
References 167

LIST OF TABLES

Table 1-1. Inflammatory markers associated with cognitive decline and dementia in epidemiologic studies 19

Table 1-2. Test scores in the ARIC MRI study among participants who completed cognitive tests on all four follow-ups 19

Table 1-3. Gingival crevicular fluid-mediator levels and Biofilm-Gingival Interface categories 19

Table 3-1. Numbers of subjects participating in the ARIC study from Visit 1 through Visit 4, Dental ARIC, and Brain MRI 49

Table 3-2. Definition and prevalence of periodontal disease classified by Biofilm-Gingival Interface (BGI) system in adults aged 52 to 74 years at ARIC Visit 4 49

Table 3-3. Summary of candidate variables, coding, and analytic approach 50

Table 4-1. Race- and gender- specific of ARIC Visit 4 characteristics of study samples 65

Table 4-2. Comparisons of self-reported causes of tooth loss, the use of prosthesis, and gum disease of study participants who were edentulous and those who were dentate 66

Table 4-3. Regression coefficients for the associations between oral health measures and Visit 4 cognitive scores 67

Table 4-4. Self-reported causes of tooth loss, prosthesis use, gum disease, oral hygiene care, and dental visits of dentally-screened participants 71

Table 4-5. Cognitive scores at Visit 4 in relation to selected study characteristics (n = 9,874) 73

Table 4-6. Study centers-specific estimates for association between oral health measures and cognitive function 75

Table 4-7. Prevalence of periodontal disease classified by CDC/AAP index and other clinical measures of periodontal disease in relation to five levels of Biofilm-Gingival Interface 76

Table 4-8. Regression coefficients for associations of CDC/AAP periodontal disease classification and clinical signs of periodontal disease with three measures of cognitive function at Visit 4 77

Table 4-9. Regression coefficients for associations between dental status (complete tooth loss *vs.* dentate) and three cognitive scores at Visit 4 (n = 9,874) 78

Table 4-10. Regression coefficients for associations between periodontal disease classified by Biofilm-Gingival Interface (BGI) classification and three cognitive scores at Visit 4 (n = 5,942) 79

Table 4-11. Regression coefficients for associations between number of teeth and three cognitive scores at Visit 4 (n = 5,942) 80

Table 5-1. Race- and gender-specific socio-demographics, health conditions, and cognitive function at baseline (1996-1998) of study participants from Forsyth County NC and Jackson MS study sites 98

Table 5-2. Cognitive scores at Visit 4 and the eight-year changes in cognitive scores 99

Table 5-3. Regression coefficients for the effects of time, oral health measures, and the interaction between time and oral health measures on three cognitive scores 100

Table 5-4. Regression coefficients for the associations between oral health measures and Visit 4 cognitive scores: A cross-sectional analysis 106

Table 5-5. Comparison of cognitive scores at baseline and follow-up of participants who were from Forsyth County NC and Jackson MS 107

Table 5-6. Study site-specific estimates for associations between time, dental status, and their interaction on three measures of cognitive function 108

Table 5-7. Regression coefficients for the effects of time, oral health measures, and their interaction on three cognitive scores, excluding subjects with stroke at baseline 109

Table 5-8. Regression coefficients for effects oral health measures on cognitive function changes: Change score models 110

Table 5-9. Baseline characteristics and repeated measures (1996-1998 and 2004-2006) of the three cognitive test scores (n = 911) 111

Table 5-10. Regression coefficients for effects of time, dental status, and their interaction on cognitive scores (n = 911) 113

Table 5-11. Stratum-specific estimates for associations between dental status and cognitive scores (n = 911) 114

Table 5-12. Regression coefficients for effects of time, periodontal disease, and their interaction on cognitive scores (n = 558) 115

Table 5-13. Stratum-specific estimates for associations between periodontal disease and cognitive scores (n = 558) 116

Table 5-14. Regression coefficients for effects of time, number of teeth, and their interaction on cognitive scores 117

Table 5-15. Stratum-specific estimates for the association between number of teeth and cognitive scores (n = 558) 118

Table A-1. Cardiovascular and other non-oral health risk factors for cognitive decline, dementia, and brain abnormalities identified in the ARIC studies 136

Table A-2. Cognitive decline and dementia are associated with an increased likelihood of poor oral health 138

Table A-3. Possible causal relationships of periodontal disease or tooth loss with cognitive decline 139

Table A-4. Clinical measures, inflammatory biomarkers, and microbiology components of periodontal diseases: Dental ARIC 142

Table C-1. Selected baseline characteristics of ARIC study samples in relation to dental status 145

Table C-2. Prevalence of periodontal diseases in Dental ARIC study, classified by CDC/AAP[1] index and Biofilm-Gingival Interface classification (n = 6,700) 147

Table C-3. Selected characteristics of ARIC study participants at Visit 4 in relation to periodontal disease prevalence: A cross-sectional study (n = 5,966) 149

Table C-4. Selected characteristics at Visit 4 of ARIC study participants from Jackson and Forsyth County study sites in relation to periodontal disease: A longitudinal study (n = 558) 150

Table C-5. Selected characteristics of ARIC study participants in relation to number of teeth 153

Table D-1. Means, standard deviations, and median scores of three cognitive tests for African American and white participants at each ARIC visit 154

Table D-2. Cognitive scores at Visit 4 in relation to selected study characteristics (n = 9,909) 157

Table E-1. Characteristics of ARIC cohort members who were included in or excluded from cross-sectional and cohort studies 163

Table E-2. Selected characteristics of ARIC study participants by study sites: A cross-sectional study 166

LIST OF FIGURES

Figure 1-1. Age-specific prevalence of all types of dementia (per 100 population) across continents and countries 20

Figure 3-1. Data sources, key variables, and study design for each specific aim 51

Figure 3-2. Power plot for detectable difference in means between BGI-DL/SB *vs*. others 51

Figure 4-1. Crude and adjusted means with 95% confidence intervals of three cognitive scores, comparing edentulous participants with dentate participants (n = 9,874) 68

Figure 4-2. Crude and adjusted means with 95% confidence intervals of three cognitive scores, comparing among five levels of periodontal conditions (n = 5,942) 69

Figure 4-3. Flow chart of ARIC participants who completed three cognitive function tests, dental screening, and comprehensive dental examination between 1996 and 1998 81

Figure 4-4. Extent of probing pocket depth by five levels of Biofilm-Gingival Interface classification (n = 5,942) 82

Figure 4-5. Extent of attachment loss by five levels of Biofilm-Gingival Interface classification (n = 5,942) 82

Figure 5-1. Crude and adjusted means with 95% confidence intervals for the Delayed Word Recall test scores at baseline and follow-up comparing edentulous with dentate participants (n = 911) 101

Figure 5-2. Crude and adjusted means and 95% confidences intervals for the Delayed Word Recall test scores at baseline and follow-up in relation to five levels of periodontal conditions (n = 558) 101

Figure 5-3. Crude and adjusted means and 95% confidences intervals for the Digit Symbol Substitution test scores at baseline and follow-up comparing edentulous with dentate participants (n = 911) 102

Figure 5-4. Crude and adjusted means and 95% confidences intervals of Digit Symbol Substitution test scores at baseline and follow up in relation to five levels of periodontal conditions (n = 558) 102

Figure 5-5. Crude and adjusted means and 95% confidences intervals of Word Fluency test scores at baseline and follow up comparing edentulous with dentate participants (n = 911) 103

Figure 5-6. Adjusted means and 95% confidences intervals of Word Fluency test scores at baseline and follow up in relation to five levels of periodontal conditions (n = 558) 103

Figure 5-7. Flow chart of ARIC participants who completed two cognitive function assessments (1996-1998 and 2004-2006) and participated in the Dental ARIC Study 119

Figure 5-8. Scatterplots for the association between baseline cognitive scores and change in the Delayed Word Recall by race, study sites, and dental status (n = 911) 120

Figure 5-9. Scatterplots for the association between baseline cognitive scores and change in the Digit Symbol Substitution scores by race, study sites, and dental status dental status (n = 911) 121

Figure 5-10. Scatterplots for the association between baseline cognitive scores and change in the Word Fluency scores by race, study sites, and dental status dental status (n = 911) 122

Figure B-1. Directed acyclic graph for associations between periodontal disease and cognitive function 143

Figure B-2. Directed acyclic graph for associations between tooth loss and cognitive function 144

Figure C-1. Extent of probing pocket depth among Dental ARIC participants, by five levels of Biofilm-Gingival Interface classification (n = 6,700) 147

Figure C-2. Extent of attachment loss of Dental ARIC participants by five levels of Biofilm-Gingival Interface classification (n = 6,700) 148

Figure C-3. Density plot for a distribution of number of teeth for Dental ARIC participants (n = 6,700) 151

Figure C-4. Boxplots for number of teeth by five levels of Biofilm-Gingival Interface (BGI) classification for ARIC participants: A cross-sectional study (n = 5,966) 152

Figure C-5. Boxplots for number of teeth by five levels of Biofilm-Gingival Interface (BGI) classification for ARIC participants: A cohort study (n = 558) 152

Figure D-1. Pearson correlation coefficients among three cognitive scores at 1996-1998 for ARIC participants (n = 11,097) 155

Figure D-2. Pearson correlation coefficients among three cognitive function scores at 2004-2006 for ARIC participants (n = 1,101) 156

Figure D-3. Race- and gender-specific density plots for three cognitive scores measured at Visit 4: A cross-sectional study (n = 9,909) 159

Figure D-4. Density plots for a) Delayed Word Recall scores at a) 1996-1998 and 2004-2006; b) race- and gender-specific changes in Delayed Word Recall scores: A cohort study (n = 911) 160

Figure D-5. Density plots for a) Digit Symbol Substitution scores at 1996-1998 and 2004-2006; b) race- and gender-specific changes in Digit Symbol Substitution scores: A cohort study (n = 911)..... 161

Figure D-6. Density plots for a) Word Fluency scores at 1996-1998 and 2004-2006; b) race- and gender-specific changes in Word Fluency scores: A cohort study (n = 911) 162

LIST OF ABBREVIATIONS

AD	Alzheimer Disease
ACT	Antichymotrypsin
APOE	Apolipoprotein E
ARIC	The Atherosclerosis Risk in Communities
BGI	Biofilm-Gingival Interface
BMI	Body Mass Index
BOP	Bleeding on Probing
CAL	Clinical Attachment Level
CDC/AAP	Centers for Disease Control / American Academy of Periodontology
CHD	Coronary Heart Disease
CI	Confidence Interval
CRP	C-Reactive Protein
DAG	Directed Acyclic Graph
DL	Deep Lesion
DWR	Delayed Word Recall
DSS	Digit Symbol Substitution
G	Gingivitis
GCF	Gingival Crevicular Fluid
GEE	Generalized Estimating Equations
GLMs	Generalized Linear Models
GR	Gingival Recession
H	Healthy
HR	Hazard Ratio
IL	Interleukin

ICAM	Intracellular Adhesion Molecule
IPWS	Inverse Probability Weights
LDL	Low Density Lipoprotein
LB	Low Bleeding
MB	Moderate Bleeding
MCI	Mild Cognitive Impairment
MMSE	Mini-Mental State Examination
MRI	Magnetic Resonance Imaging
MSM	Marginal Structural Model
NHANES	National Health and Nutrition Examination Survey
NSAIDS	Nonsteriodal Anti-Inflammatory Drugs
PGE_2	Prostaglandin E_2
PPD	Probing Pocket Depth
PR	Prevalence Ratio
OR	Odds Ratio
WF	Word Fluency
WMHs	White Matter Hyperintensities
SB	Severe Bleeding
SD	Standard Deviation
SE	Standard Error
SW	Sulcal Width
TNF	Tumor Necrosis Factor
VaD	Vascular Dementia
VCAM	Vascular Cell Adhesion Molecule

VIF	Variance Inflation Factor
VZ	Ventricular Size

LITERATURE REVIEW

A. Introduction

Dementia and cognitive impairment are major public health problems that are of growing concern in aging populations. Affected individuals become more dependent as cognitive impairment and dementia progress, disrupting their personal lives and those of their caregivers, as well as causing substantial expenditures for medical and long-term care services (1). At present, effective prevention or treatment is unavailable, and biological pathways contributing to cognitive impairment and dementia are not clearly understood.

B. Dementia and cognitive decline

Age-related cognitive decline, mild cognitive impairment, and dementia

Cognitive decline is a decrease in the ability of the brain to perform regular functions such as judgment, reasoning, memory, learning, and understanding. Decline can be a result of neurodegenerative disease or the aging process (2). Neurodegenerative diseases that cause cognitive decline include Alzheimer disease (AD) and vascular dementia (VaD) (e.g., due to stroke). Cognitive aging is a result of aging-related changes in brain physiology, the cerebrovascular system, and neurochemical levels. The changes produce decreases in brain vascularization, reduction in neurotransmitter levels, and general brain atrophy (3). Mild cognitive impairment (MCI) is a level of cognitive decline which, although it does not affect the ability to lead an independent life, is more pronounced than cognitive aging. Cognitive decline and MCI can occur at any age but primarily affect the elderly. About 10% to 20% of people aged 65 years and older have MCI (1).

Older adults with MCI are at increased risk of developing dementia, a chronic and irreversible deterioration of cognition. Education is associated with lower rates of age-related cognitive decline because a high level of education may be a proxy indicator of cognitive reserve. Higher education level reflects better resilience and plasticity of cognitive networks that protect individuals from the negative effects of aging (3). Moreover, education attainment is related to risk factors for the underlying pathologic processes of dementia, such as lifetime patterns of health care use and health behaviors. Each year, about 10% to 15% of individuals with MCI are estimated to progress from MCI to dementia. In one study, progression rates from MCI to dementia were 2.3, 1.3, and 0.3/100 person-years for Alzheimer disease, vascular dementia, and other types of dementia, respectively (4). As the aging population is growing, attempts to identify MCI and predictors of progression from MCI to dementia become important, because MCI is treatable. Furthermore, the earlier the interventions occur, the greater the possibility to delay or stop the neurodegenerative process. Many studies, therefore, use brain imaging techniques and biomarkers in addition to neuropsychological testing to identify people who are likely to progress from MCI to dementia (5-7).

Public health burden of cognitive decline and dementia

Cognitive decline and dementia are major public health problems affecting older adults in the United States (U.S.) and worldwide. Incidence and prevalence of dementia rise substantially with age (1). In the U.S., the prevalence of all types of dementia ranges from approximately 2% in people aged 65-69 up to more than 30% in people over 90 years of age (Figure 1-1). Incidence rates are between approximately 4 per 1000 person-years in people aged 65-69 and up to 60 per 1000 person-years among the group aged over 90 years old (8). The two most common types of dementia are AD and VaD, accounting for up to 75% of all dementia cases (4,8,9).

As aging populations are growing and effective treatment and prevention are lacking, the costs and social burden associated with dementia are increasing. The number of people in the U.S. with AD will be as high as 13.5 million by 2050, almost three times the number in the year 2000 (10).

Furthermore, the 2012 report revealed that annual health care costs for patients with AD and other dementias were triple the health care costs for adults aged 65 and older without dementia (1). At the individual level, dementia results in physical disability, institutionalization, decreased quality of life, and increased risk of mortality. Therefore, it is essential to identify underlying mechanisms and treatable factors affecting clinical onset and progression of dementia.

C. Determinants of cognitive decline, dementia, and structural brain abnormalities

Advancing age and low education are prominent risk factors for age-related changes in cognitive function (1,3,8). Many observational studies have identified potential risk factors that may contribute to initiation and progression of dementia and cognitive decline, such as severe atherosclerosis, hypertension, diabetes mellitus, hyperlipidemia, and smoking (6,8,11-13). However, few factors have sufficient evidence to support a causal association with cognitive decline and dementia. Stroke and cardiovascular risk factors are consistently reported as risk factors for neurocognitive disease, particularly VaD. Several studies have also related genetic factors as well as systemic infection and inflammation to both AD and VaD (7,14-16). However, specific underlying mechanisms of AD and VaD have not been clearly characterized (8,9). Neurodegenerative changes (i.e., progressive brain atrophy and accumulations of cortical senile plaques and neurofibrillary tangles) in AD and cerebral infarction in VaD may lead to neuronal or axonal loss, impairing cognitive function. Since AD and VaD are the two most prevalent dementia subtypes, the following is a summary of major risk factors that may contribute to cognitive impairment caused by AD or VaD pathogeneses. Relevant findings from ARIC studies are summarized in Table 7-1.

APOE genotype

Despite uncertainty about the causes of cognitive impairment and dementia, genetic factors are accepted as part of the etiology, particularly the association between the apolipoprotein E (APOE) ε4 allele and sporadic AD (late-onset AD) (8,9,17). A higher frequency of the APOE ε4 allele was

found in demented compared to non-demented people, with an apparent dose-response relationship (16,18). Compared to individuals with the ε3/ε3 genotype, the risk of dementia hospitalization for persons with the ε4/ε4 genotype was highest, followed by those with ε3/ε4 genotype (19). Several longitudinal studies have shown a greater cognitive decline among non-demented people who are carriers of the APOE ε4 allele (11,14).

In the ARIC cohort, associations between the APOE genotype and cognitive decline among middle-aged white participants have been reported. The greatest cognitive decline was found in participants with combinations of APOE ε4 with cardiovascular risk factors, particularly hypercholesterolemia or diabetes, suggesting a synergistic effect (14). Likewise, in a Dutch study, AD and VaD were associated with atherosclerosis, and the association was pronounced in participants with APOE ε4 (20).

The relationships of APOE ε4 with cognitive decline and dementia are well-documented (18-20); however, the underlying mechanisms are unknown. A possible mechanism is that APOE enhances proteolytic breakdown of peptide beta-amyloid protein in the brain, both within and between cells, and the isoform APOE ε4 is not as efficient as other isoforms in catalyzing this reaction. Consequently, individuals with the APOE ε4 variation tend to accumulate beta-amyloid protein, predisposing to AD. In addition to an increased risk of AD, APOE ε4 also increases risk of cardiovascular disease as a result of impaired cholesterol transport and metabolism (18,21).

Stroke and cardiovascular risk factors

Cross-sectional and longitudinal studies, including the ARIC study, have reported that stroke, coronary heart disease (CHD), and cardiovascular risk factors are related to a higher risk of dementia in elderly people (6,11,19,22). As for genetic factors, specific biological mechanisms linking these environmental factors to neurocognitive disorders remain unclear. Multiple infarcts and small vessel atherosclerosis in the brain caused by these risk factors can lead to subsequent cognitive decline and

VaD (16). The observed associations among stroke CHD, and cognitive decline may also be due to sharing several common risk factors such as low education, smoking, and diabetes mellitus (8,12,16,22).

Stroke: Stroke causes cognitive and motor impairments that are both acute and chronic. History of stroke is also a risk factor for developing dementia (16). In ARIC, a follow-up study conducted over a 14-year period found that stroke was associated with a decline in performance on the DWR and WF test scores but not on the DSS test (11).

CHD: Evidence from studies examining an association between CHD and cognitive performance is inconsistent. Two large epidemiologic studies have reported an association between CHD and lower cognitive scores (22,23) Another study with fewer participants did not observe the association between CHD and dementia diagnosis (24). Low cardiac output, brain hypoperfusion, and cardiac microembolization after myocardial infraction have been proposed as possible causal pathways of cognitive impairment.

Hypertension: Hypertension in midlife is a strong risk factor for cognitive decline, cerebral abnormalities, and dementia (12). In the ARIC cohort, baseline hypertension was associated with a decline only in the DSS test score (25).

Diabetes: Studies have shown a clear association of diabetes with both cognitive decline and dementia (19). People with diabetes at baseline exhibited greater decline in cognitive function over the six-year interval. Of the three cognitive tests (DWR, DSS, and WF), change in the DSS scores was most strongly associated with diabetes (25). Associations between clinical signs or symptoms related to diabetes and cognitive disorders, however, were inconsistent (26,27). Cognitive decline was not correlated with hyperglycemia (26), but a relation was detected with hyperinsulinemia (27).

High serum cholesterol: Many studies fail to show an association of elevated low-density lipoprotein (LDL) cholesterol or triglycerides with dementia, cognitive impairment, or abnormalities on brain imaging (4,28). Similarly, elevated LDL was not associated with cognitive decline in

middle-aged adults after follow-up for 6 years in the ARIC study (25). Nonetheless, hypercholesterolemia, elevated total cholesterol in midlife, was associated with dementia incidence in a longitudinal study with an average follow-up of 12.8 years (19).

Smoking: A cross-sectional study reported a dose-response relation between cigarette smoking and silent cerebral infarction, in which infarction prevalence was highest for current smokers, followed by ex-smokers, persons exposed only to environmental tobacco smoking, and nonsmokers (29). A 14-year prospective study of the ARIC cohort found smoking to be the primary risk factor for dementia incidence (19). However, in another prospective study with a follow-up interval of 6 years in the same cohort, the most important risk factors for cognitive decline were diabetes and hypertension; the association of cognitive decline with cigarette smoking was not significant (25).

Alcohol consumption: A previous study has shown a protective effect of low and moderate alcohol consumption on stroke (30). In the ARIC study, current drinkers had higher baseline scores for cognitive performance than nondrinkers (31). Alcohol intake was not associated with the presence of brain infarction lesions. However, a positive correlation between number of alcoholic drinks per week and brain atrophy was observed (32).

Systemic infection and inflammation

Viral or bacterial pathogens and inflammation have the potential to cause neurocognitive disease; however, the specific mechanisms are unknown. Several types of data including post-mortem examinations have suggested that chronic infection and inflammation might contribute to the neurodegenerative process leading to dementia. High levels of inflammatory mediators (e.g. serum C-reactive protein (CRP) and Interleukin-6 (IL-6)) have been linked to an increased risk of cognitive decline and dementia in epidemiological studies (7,15,33-40) (Table 1-1). Pro-inflammatory factors derived from immune responses to local chronic infection may reach the brain via the systemic circulation, exacerbating inflammatory processes or vascular pathologies (41). Herpes virus, bacteria

(e.g. *C. pneumoniae*), and periodontal pathogenic spirochetes (e.g., *T. denticola, T. pectinovorum, T. vincenti, T. amylovorum, T. maltophilum, T. medium, and T. socrankii*) have been detected in the brains of demented patients, suggesting other possible risk factors for AD (42-44). Observational studies have also shown the benefits of using long-term, nonsteroidal anti-inflammatory drug (NSAIDs) to prevent or slow cognitive decline; however, results from clinical trials have failed to confirm any positive effect on cognition (7,13).

D. Associations of oral health measures with cognitive function

Public health burden of periodontal disease and tooth loss in adults

Periodontal disease is defined as a chronic infectious disease that causes inflammation of periodontal tissues and destruction of the periodontal ligament that attaches the tooth to its bony socket. Periodontal disease is associated with increased systemic inflammatory markers such as CRP and local inflammatory levels such as gingival crevicular fluid (GCF) prostaglandin E2 (PGE_2) (17,45). As a result of inconsistent case definitions for PD, there is a difficulty in comparing the disease prevalence and incidence estimates across epidemiologic studies (46). Overall, prevalence of periodontal disease has declined in the U.S. population. The Third National Health and Nutrition Examination Survey: NHANES III (1998-1994) estimated that 10% of adults (20-64 years) had moderate or severe periodontal disease compared with 5% in 1999-2004[1], using the same case definition. Moderate or severe periodontitis decreased from approximately 27% to 17% between 1988-1994 and 1999-2004 among adults aged 65 years and older (47). However, these data, which

[1]The Centers for Disease Control and Prevention/ The American Academy of Periodontology (CDC/AAP) case definitions were applied. Severe periodontitis was defined as the presence of 2 or more interproximal sites with ≥ 6 mm attachment loss (not on the same tooth) and 1 or more interproximal site(s) with ≥ 5 mm pocket depth . Moderate periodontitis was defined as 2 or more interproximal sites with pocket depth ≥ 4 mm attachment loss (not on the same tooth) or 2 or more interproximal sites with pocket depth ≥ 5 mm, also not on the same tooth. Mild periodontitis was defined as ≥ 2 interproximal sites with ≥ 3 mm attachment loss and ≥ 2 interproximal sites with ≥ 4 mm (not on the same tooth) or 1 site with ≥ 5 mm pocket depth.

obtained using a partial-mouth protocol (i.e. one maxillary and one mandibular quadrant), underestimate true prevalence observed when all periodontal tissues are assessed (48).
A recent NHANES (2009-2010), which is the first national survey to use a full mouth protocol, reported that total prevalence of periodontitis ranged from 24.4% in adults 30-34 years old to 70.1% in adults aged 65 years and older. Prevalence of mild, moderate, and severe periodontitis in adults aged 30 years and older was 8.7%, 30.0%, and 8.5%, respectively (49).

Advanced periodontal disease destroys the periodontal ligament, loosening the tooth and contributing to tooth loss. Tooth loss has been described as a clinically-meaningful outcome of dental caries and periodontal disease (50), although in all age groups, dental caries is a more common underlying cause of tooth loss (51). Complete tooth loss (edentulism) is common among older adults aged 65 or older worldwide. The positive association with age can be attributable primarily to elevated rates of tooth loss in generations born in the first half of the 20th century (52). At present, prevalence of complete tooth loss is declining in the U.S. For adults (20-64 years) the prevalence of edentulism decreased from approximately 6% during 1988-1994 to 4% during 1999-2004. For older adults (65-74 years), complete tooth loss was 24% in 1999-2004 compared with 29% in 1988-1994 (47). The recent NHANES (2009-2010) reported that 15% of adults 65-74 years old and 22% of adults 75 years and older were edentulous (53). Tooth loss affects not only chewing ability and nutritional status (54,55), but also quality of life and self-sufficiency (21,56). Associations of tooth loss with an increased risk of stroke, mortality, and elevated inflammatory markers have also been shown (57,58).

Bidirectional associations between oral health measures and cognitive function

A number of both cross-sectional and longitudinal studies suggest an association between cognitive decline and poor dentition in the elderly (59-64), but it is not well established if the same relationship exists in middle-aged adults (65). The observed association may be confounded, since poor oral health and cognitive impairment share several common risk factors, such as low

socioeconomic status, smoking, and diabetes mellitus. Furthermore, the association likely is bidirectional and the underlying mechanisms remain unclear.

There are several proposed explanations as to how cognitive decline might contribute to dental caries, severe periodontal disease, and tooth loss (66,67). First, older adults with impaired memory and reduced physical function lack the capacity to perform proper oral health care (66). Second, older individuals are less likely to receive regular dental care (68). Third, decreased saliva production is a side effect of many commonly prescribed medications used to treat depressive symptoms in demented people, thereby impairing oral clearance and neutralization of dental plaque acid (69). Recently, two large observational studies suggest that socioeconomic inequalities in oral health might explain the relationship between cognitive ability and oral health. The authors proposed that low cognitive ability limits education achievement and income, and thus impairs self-care, which may lead to poor oral health (60,70).

Poor oral health resulting from cognitive impairment has been confirmed in many studies, but several findings have suggested that the reverse association is plausible. Periodontal disease and tooth loss in midlife may lead to early onset and rapid progression of cognitive decline (59,61,71). A longitudinal study of aging and AD suggested that a low number of teeth (0-9) was related to increased prevalence and incidence of dementia (72), and the decline in cognitive function was more rapid for those who carried the APOE ε4 allele (73). A case-control study in monozygotic twins discordant for probable AD showed that tooth loss early in life was associated with increased risk for dementia after adjusting for socioeconomic status. In that study, tooth loss occurred years before the diagnosis of AD, suggesting that oral disease and possibly antecedent PD might hasten progression of AD (74). Additionally, a case-control study showed that PD was a cause of tooth loss in most AD cases and occurred 20-30 years before dementia (75).

Several biologically plausible pathways have been proposed for a causal effect of oral disease on cognition. However, most evidence comes from cross-sectional studies (65,71,76,77), longitudinal studies with small sample size (61,73,78,79), and varieties of periodontal case definitions and cognitive tests (Appendix A). Potential mechanisms include inflammatory mediators produced in response to periodontal pathogens (76), dissemination of gram-negative bacteria to the brain, and increased risk of stroke and cerebrovascular injury (17,80-83).

Contribution of periodontal disease and tooth loss to cognitive decline and dementia

Periodontal pathogens can induce systemic inflammation. Previous studies have showed the involvement of inflammation in AD, though it remains unclear whether the inflammatory process is related specifically to initiation or progression of the disease. Elevation of inflammatory biomarkers, such as serum CRP, has been associated with periodontal pathogens, periodontitis, and dementia (76,80,82,84). NHANES III revealed a positive association between systemic exposure to *P. gingivalis* (measured as serum antibody to *P. gingivalis*) and poor cognition (85). Thus, it has been hypothesized that periodontal pathogens may influence neuropathogenesis of dementia via the inflammatory process (41). Lipopolysaccharide, a bacterial endotoxin, from periodontal pathogens can stimulate proinflammatory cytokines as well as CD14 activity, resulting in chronic elevation of systemic inflammatory markers. In addition, chronic periodontitis may result in long-term locally increased proinflammatory molecules that surround the trigeminal cranial nerve endings (6). Hypothetically, then, periodontal-derived cytokines could reach the brain by both systemic and neural pathways and amplify brain cytokine pools, contributing to the progression of dementia (33,41,81).

Periodontal pathogens can invade brain tissue. Periodontal pathogens such as *P. gingivalis, T. denticola, and A. actinomycetemcomitans* are capable of invading multiple cell types. These bacteria have been found at distant sites, including atherosclerotic plaque and brain tissue (86). The Treponema species has been detected in the trigeminal ganglia, brainstem, and cortex of human brain. Several studies have shown that the brains of AD patients were more likely than controls to

have Treponema (42-44). These finding suggested that oral bacteria may be capable of invading brain tissue via peripheral nerve fibers.

Periodontal disease may increase risk for CHD and stroke. Previous studies discussed several potential mechanisms relating periodontal disease and CHD, including bacteremia, systemic inflammation, and vascular injury (45,86). Periodontal pathogens have been found in atherosclerotic plaques, and they may induce vascular pathology through their cytotoxicity or inflammatory process. It has been shown that chronic, low-grade inflammation in response to periodontal infection may contribute to the development of atherosclerosis or CHD (45,83). Systemic inflammatory biomarkers, which are elevated in people with PD and CHD, include CRP, IL-6, tumor necrotic factor-alpha (TNF-α), and fibrinogen (45,87). Since PD is associated with elevated risk of stroke and CHD (83,87), which are risk factors for dementia (4,22), the elevated risk of dementia among people with periodontal disease may be mediated by CHD and stroke. Nonetheless, a recent review by Lockhart *et.al*. (2012) concluded that although current evidence suggests an independent association between periodontal disease and cardiovascular diseases, the evidence is insufficient to demonstrate the causal relationship (88).

Tooth loss results in malnutrition. Weight loss and deficiency of vitamin B6 have been suggested as potential risk factors for dementia (8,89). Tooth loss can lead to impaired masticatory function, thereby influencing food choices and nutritional status. However, a review study found that most studies reported a weak association between tooth retention, masticatory function and nutrition, and thus a definite casual effect cannot be established (90). In an animal model, the loss of molar teeth resulted in reduced mastication and impairment of spatial memory (91,92).

In summary, the association between poor oral health and cognitive impairment is complex and likely bidirectional, with poor dental health both a risk factor for and a consequence of cognitive impairment. To clarify the link between oral health and cognitive function will require a consistent

body of evidence from longitudinal studies with standardized measures of periodontal disease and tooth loss as well as careful follow-up. From a public health perspective, if further studies consistently identify periodontal disease as a risk factor for cognitive decline and dementia, the implications are significant since periodontal disease is treatable and preventable. Therefore, epidemiological studies investigating the relationship of periodontal disease as well as tooth loss with cognitive function are warranted.

E. Cognitive assessments

Changes in cognitive function may occur in one or more cognitive domains and those domains may change differentially within an individual (2). Cognitive screening assessments aim to objectively assess a patient's history of cognitive deficits or chief symptoms (3). Many instruments and approaches are available to screen for cognitive impairment, but none covers all cognitive domains (93). In addition, patterns of impairment differ among subtypes of dementia, and thus, no single test can accurately screen for all forms of dementia. Most instruments focus on memory impairment, a common symptom of AD at the initial stage of disease. In addition to memory loss, AD can manifest as gradually progressive neuropsychological deficits with, for example, dysphasia or subtle abnormalities in executive function. A well-known instrument for AD screening, the Mini-Mental State Examination (MMSE), is a brief and focused screen of cognitive domains most often affected in AD. This test includes items such as asking patients to name actual time and place of the test, repeat lists of words, perform arithmetic (e.g., serial sevens), and perform tasks involving language use and comprehension as well as basic motor skills. Possible scores range from 0 to 30, and scores between 21 and 24 indicate MCI. Since MMSE is very limited in evaluating executive function, additional tests for attention, language, praxis, visuomotor functioning, abstract reasoning, and executive functioning have been suggested as accompaniments to MMSE to improve screening sensitivity (3,94).

Each dementia subtype is characterized by different cognitive domain deficits. In comparison with people with AD, those with VaD tend to score lower on tests of executive function, such as verbal fluency, and their level of memory impairment is usually less severe. Similarly, people with frontotemporal dementia typically possess reduced letter fluency and executive function than people with AD, but their memory performance is often better. Individuals diagnosed as Lewy body dementia are even more dysfunctional in areas such as attention, visuospatial tasks, letter fluency, mental tracking, and abstract reasoning. Therefore, a comprehensive screening instrument should cover the following six core domains: a) attention/working memory; b) new verbal learning and recall; c) expressive language; d) visual construction; e) executive function; and f) abstract reasoning (93).

There is no consensus regarding a standard screening test; using both formal and informal assessments may provide more information about people's cognitive performance. A formal test provides a summary measure of cognitive performance, but that measure may be influenced by anxiety on the part of the subject. An informal evaluation, carried out through a conversation using simple questions, may permit a more realistic assessment of cognitive performance in relation to cognitive deficits, but will not provide a summary score.

Cognitive function assessment in the ARIC study

The entire ARIC cohort completed two neurocognitive function assessments at both Visits 2 and 4 (Figure 3-1). For those who participated in the Brain MRI study, cognitive function assessments were also carried out at Visit 3 and once between 2004 and 2006. Three standard cognitive tests (DWR, DSS, and WF) administered at every follow-up visit were used to quantify changes in cognitive function. The DWR measures verbal memory, whereas both the DSS and WF assess executive function. Higher test scores indicate better cognitive performance. Recently, the ARIC cohort has been undergoing the third cognitive function assessment (Visit 5), which included more neurocognitive tests. A total of seven core domains are examined: memory (verbal and

nonverbal), language, visuospatial, attention, executive function, motor function, and premorbid intelligence. However, Visit 5 data collection was not complete at the time of writing this dissertation.

A previous ARIC study reported that DWR, DSS, and WF scores at baseline were normally distributed and negatively associated with age (31). Participants aged 65-69 years had lower cognitive scores compared to those who were younger, and women had higher average scores than men for every age group. In addition to demographic characteristics, baseline cognitive function was also associated with cardiovascular risk factors, including smoking status, alcohol use, depressive symptoms, diabetes, and hypertension. A subset of ARIC participants was followed for fourteen years. An analysis found that stroke, diabetes, hypertension, metabolic syndrome, and APOE genotype predicted cognitive decline. However, the magnitude of changes for the DWR, DSS, and WF scores was relatively small (11) (Table 1-2).

F. Neuroimaging

Neuroimaging provides a sensitive and noninvasive method for detecting subclinical abnormalities in both cortical and subcortical brain structures. With the introduction of MRI, a number of recent studies have focused on assessing brain structural changes in normal aging, especially hippocampal-parahippocampal atrophy to detect early AD and MCI (5,95). Positive correlations between variation in brain structure and cognitive function have also been shown in healthy adults, particularly in domains such as processing speed, executive function, and memory (5,96). However, the basis for cerebral-cognitive relationships observed in current studies remains unclear.

White matter hyperintensities (WMHs), which appear as “bright signals” on the MRI image, are common MRI findings that reflect demyelination, a deterioration of neural pathway. WMHs been associated with cognitive deficits in both AD and normal aging (96,97). White matter consists of glial cells and myelinated axons that are important for signal transmission in the nervous system.

Atherosclerosis of arteries and arterioles supplying blood to white matter may cause neurovascular changes as well as a reduction in myelination. The degradation and disruption of these white matter pathways will then result in cognitive deficits. Greater brain volume has been thought to be a protective factor for neurocognitive disorders, including cognitive aging. Ventricular and sulcal size as measures of brain volume have been associated with reduced cognitive function in both normal aging and AD (5,96,98). A longitudinal study in AD patients suggested that greater ventricular size measured on MRI might be a marker for preclinical AD neuropathology (98). Several pathologies such as neuronal loss, brain tissue density reduction, white matter degeneration, and microvascular pathology can result in decreased brain volume or brain atrophy (5,95,97).

Brain MRI assessment in the ARIC study

A study using ARIC Visit 3 data reported prevalence of brain neurodegenerative changes by comparison subjects' MRI images with standardized images that successively increased from barely detectable (score 1) to extensive change (score 8). Studies with no change received score 0, and those with changes worse than score 8 received score 9. A high-grade abnormality was defined as ventricular grade 4 or higher (14%), sulcal size grade 3 or higher (26%), and WMHs grade 3 or higher (11%). Associations between these abnormalities and cognitive test scores have been reported (high grade WMHs with DWR, DSS, and WF; high ventricular grade with DWR and DSS; and high sulcal grade with DWR scores) (96). Two other ARIC studies reported that brain atrophy was correlated with diabetes (99) and alcohol consumption (32). Although they did not find an association of brain atrophy and hypertension, Knopman *et al*. (2005) did find a strong association between hypertension and the level of WMHs (99). Similar to findings from other studies, diabetes was a risk factor for brain atrophy in a 10-year follow up of this cohort. Worsening of sulcal widening was associated with diabetes, and incident infarcts were associated with both diabetes and hypertension (12). It is possible that microvascular effects of diabetes may result in decreased cerebral perfusion and subtle microinfarction, which can in turn lead to brain volume loss and cognitive decline (99).

G. Periodontal disease assessments

Clinical measures used to define periodontal disease

Previous literature reviews highlighted methodological problems in studying periodontal epidemiology, particularly a lack of uniform criteria used to define periodontitis (48,100,101). Most periodontal indices are developed by using clinical signs and symptoms of gingival inflammation, such as bleeding on probing (BOP) and destruction of periodontal supporting tissue, as measured by alveolar bone loss or attachment loss (AL)[1]. Other clinical measures that may be included are gingival recession (GR) and pocket probing depth (PPD[2]). Attachment loss is considered to be the gold standard for measuring the history of periodontal disease and its progression, as opposed to current disease activity (102). Inconsistencies in the use of disease indicators and a threshold for quantifying severity of periodontitis lead to multiple case definitions of periodontal disease. These methodological issues also arise from a difference in areas of the mouth surveyed (e.g., full-mouth, partial-mouth, or index teeth), periodontal probes, and techniques used to measure PPD and AL (100,101,103). As a result, it is very difficult to compare PD occurrence across studies.

Centers for Disease Control/ American Academy of Periodontology (CDC/AAP) classification

In 2003, a working group appointed by the CDC and AAP developed a standardized clinical case definition for population-based studies of periodontitis based on measurements of PPD and AL. The case definition for moderate periodontitis requires at least two sites on different teeth have interproximal AL > 4 mm or interproximal PPD > 5 mm. Severe periodontitis was defined as two or more interproximal sites with AL ≥ 6 mm, not on the same tooth, and at least one interproximal site with PPD ≥ 5 mm.(104). Nonetheless, validity of prevalence or incidence estimation largely depends on examination protocols, where the full mouth approach serves as “a gold standard”. A recent

[1]AL is the distance in millimeters form the cemento-enamel junction (CEJ) to the base of sulcus or periodontal pocket.

[2]PPD is the distance in millimeters from the gingival margin to the base of the gingival sulcus or periodontal pocket.

validation study demonstrated that partial mouth protocols using the CDC/AAP definition underestimated the prevalence of severe and moderate periodontitis by 63% and 59%, respectively (48). Using this case definition, prevalence of severe periodontitis in Dental ARIC was 16.9% (83). The Dental ARIC study used the full mouth protocol to measure periodontal disease in the ARIC cohort. Thus, the estimated periodontal disease prevalence is unlikely to be underestimated due to the examination protocol.

Biofilm-Gingival Interface (BGI) index

Unlike attachment loss or tooth loss that are historical markers of periodontal disease and treatment, the BGI index has been developed based on a concept that clinical disease classification should reflect an underlying biological process of periodontal disease that involves a complex interaction of the microorganisms with host inflammatory and immune response. Two clinical signs of disease, PPD and BOP, were used to create this case definition. The concept underlying the BGI is that periodontal disease represents pathology at the biofilm-gingival interface, which is bordered on the gingival tissue side by the epithelium and subgingival plaque within the pocket. Five levels of BGI were defined based on the extent of PPD (e.g., no periodontal pockets; PPD ≤ 3 mm *vs*. had periodontal pockets; PPD ≥ 4 mm) in combination with bleeding scores (e.g., low, LB; moderate, MB; or severe, SB) (Table 3-2). A study showed that levels of inflammatory mediators within the periodontal pockets (e.g., Interleukin 1-beta (IL-1β) and PGE_2) were lowest among those with healthy periodontal tissue and increased significantly among subjects with gingivitis and periodontitis. The higher levels of gingival crevicular fluid (GCF) inflammatory markers were related to severe BOP conditions. In addition, the presence of periodontal pathogens was associated with each BGI group. For example, *C. rectus* was associated with gingivitis, BGI-DL/MB and BGI-DL/SB; *P. gingivalis* was associated with BGI-deep lesions (17). In the present study,BGI index was used as a measure of periodontal disease.

Gingival crevicular fluid (GCF) inflammatory markers

Evidence from epidemiological and microbiological studies reveal that microorganisms associated with periodontal disease are also found in healthy individuals and at sites where periodontal disease is not progressing. Moreover, an individual's disease severity or extent is not associated with levels of plaque control. These findings led to the current concept that periodontal disease progression is highly dependent upon inflammatory host response to localized microbial plaque challenge (17,105,106). Lipopolysaccharide, a cell-wall component of gram negative bacteria, triggers monocytes to release inflammatory mediators that increase local destruction of the periodontium. Therefore, levels of monocyte inflammatory mediators in GCF, such as PGE_2, IL-1β, IL-6, IL-8, TNF, and collagenase can be markers of periodontal disease activity at the site level. It has been demonstrated that GCF composition reflects the nature and amplitude of the inflammatory host response to bacterial plaque and periodontal status (17,102,107). A systemic host response to periodontal disease is evidence by serum antibodies to common oral bacteria such as *P. gingivalis* (85,106), and elevation in serum inflammatory markers such as serum CRP and sICAM (17,80,82,83).

The Dental ARIC study reported levels of GCF-inflammatory markers in association withBGI index (Table 1-3). Compared to healthy subjects (BGI-H), all four categories had significantly increased GCF-levels of IL-1b and PGE_2. Increased expression of the GCF-inflammatory markers appeared to be related to the extent of gingival bleeding among individuals PPD ≤ 3 mm or PPD ≥ 4 mm (17). Inflammatory response to chronic local infection may trigger a more generalized systemic response in tissues remote from the periodontium (e.g., the liver), thereby increasing systemic levels of inflammatory markers, which may impair neurogenesis or damage existing neurons in the brain. Consequently, people with elevated levels of inflammatory markers may at an increased risk of cognitive decline.

H. Tables

Table 1-1. Inflammatory markers associated with cognitive decline and dementia in epidemiologic studies

Study	n	Inflammatory markers	Outcomes
Framingham study (35)	619	IL-1 Tumor necrosis factor alpha (TNF-α)	Dementia
MacArthur studies (36)	779	IL-6	Cognitive decline
Health ABC study (37)	3,031	IL-6 CRP TNF-α	Cognitive decline
Rotterdam study (38)	188 cases 727 controls	IL-6 CRP α-1 antichymotrypsin (ACT)	Dementia
Leiden 85+ study (39)	599	TNF-α IL-10	Cognitive decline
Honolulu-Asia Aging study (33)	1,050	CRP	Dementia
Longitudinal aging study Amsterdam (40)	1,284	α-1 antichymotrypsin (ACT)	Cognitive decline
Greek community (15)	37 cases 33 controls	CRP Intracellular adhesion molecule (ICAM-1) Vascular cell adhesion molecule (VCAM-1)	Cognitive decline

Table was modified from Watts A, Crimmins EM, Gatx M. Inflammation as a potential mediator for the association between periodontal disease and Alzheimer's disease. Neuropsychiatric Disease and Treatment 2008;4(5):865-76.(81)

Table 1-2. Test scores in the ARIC MRI study among participants who completed cognitive tests on all four follow-ups

Cognitive assessment	Visit 2 (1990-1992)	Visit 3 (1993-1995)	Visit 4 (1996-1998)	2004-2006
Delayed word recall	6.6 (1.4)	6.6 (1.6)	6.7 (1.5)	6.0 (1.7)
Digit symbol substitution	41.1 (13.2)	40.7 (14.4)	40.4 (14.0)	36.7 (13.2)
Word fluency	33.3 (12.1)	33.2 (12.8)	33.4 (12.8)	31.6 (12.3)

Means (standard deviations) of test scores for 1,018 participants.
Table was adapted from Knopman DS, Fourteen-year longitudinal study of cardiovascular risk factors, APOE genotype, and cognition: The ARIC study. Alzheimer's & Dementia 2009(5):207-14. (11)

Table 1-3. Gingival crevicular fluid-mediator levels and Biofilm-Gingival Interface categories

GCF-mediator (ng/mL)	PPD ≤ 3 mm		PPD ≥ 4 mm		
	BOP ≤ 10%	BOP >10%	BOP ≤10%	10%< BOP <50%	BOP ≥50%
	BGI-H	BGI-G	BGI-DL/LB	BGI-DL/MB	BGI-DL/SB
IL-1b , mean ± SE	104.1 ± 4.8	148.7 ± 4.7	122.5 ± 4.3	141.4 ± 2.9	194.7 ± 5.3
PGE_2, mean ± SE	198.9 ± 6.3	249.0 ± 6.3	218.1 ± 5 .3	234.7 ± 3.6	254.4 ± 6.8

Table was modified from Offenbacher S, Barrons SP, Singer RE, *et.al.* Periodontal Disease at the Biofilm-Gingival Interface. J Periodontal 2007;78;1911-1925.(17)

I. Figures

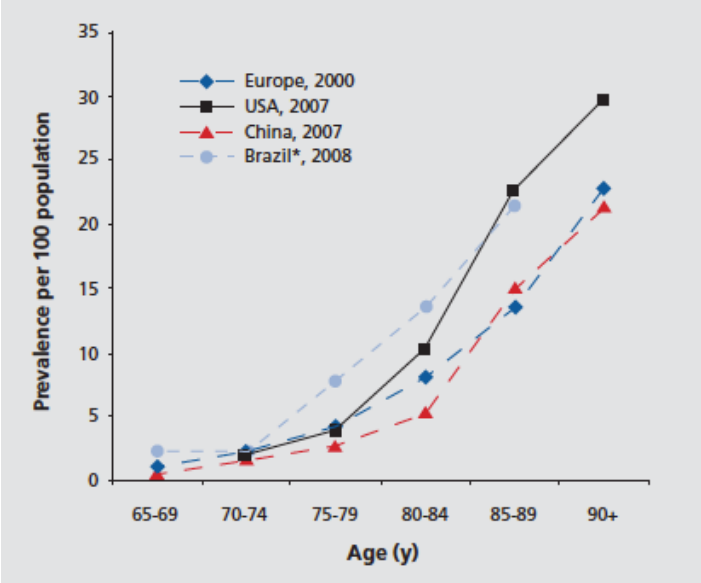

Figure 1-1. Age-specific prevalence of all types of dementia (per 100 population) across continents and countries (8)

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SPECIFIC AIMS

A. Rationale

Maintenance of cognition is important to preserve good health and quality of life, particularly in adults with advancing age. Knowledge concerning etiology and associated risk factors of cognitive decline and dementia is accumulating, but biological mechanisms remain uncertain. Several studies have reported associations of cognitive decline and certain inflammatory markers with vascular diseases and stroke (1-12). In addition, a link has been shown between impaired cognition and periodontitis, a common chronic infection associated with elevations of systemic inflammatory markers (13-15). It has also been proposed that chronic periodontal disease can contribute to early onset and rapid progression of cognitive decline (13,15,16). At present, however, there is evidence to show that there may be a reversed causal relationship, meaning that a decline in cognition can lead to poor oral health in older adults (17-19). If infection and inflammation from periodontal disease are associated with an increased risk of cognitive decline, periodontal disease can be a pivotal modifiable risk factor to delay onset or slow progression of cognitive decline among the aging population, since periodontal disease is treatable and preventable.

Hypotheses for the present study were developed based on a conceptual framework that systemic inflammation related to periodontal disease could be a potential biological pathway by which periodontal disease can lead to cognitive decline. We hypothesized that poor oral health was cross-sectionally associated with cognitive decline in late middle-aged adults. We also hypothesized that poor oral health in midlife was associated with progressive cognitive decline with advancing age.

B. Specific aims

Aim 1: Estimate associations of oral health status measures (i.e., dental status, tooth loss, and periodontal with cognitive function[1] at ARIC Visit 4.

Hypotheses: a) complete tooth loss or fewer teeth is associated with lower scores of cognitive function, and b) severe periodontal disease is associated with lower scores of cognitive function.

Overview: We evaluated the associations of oral health measures with the three cognitive tests (DWR, DSS, and WF) in late-middle aged adults after controlling for socio-demographic characteristics. We also assessed whether diabetes mellitus was an effect modifier of the associations. Participants included the subset of the ARIC cohort who completed cognitive assessment and dental screening or comprehensive dental examination at Visit 4 (1996-1998).

Rationale: An association between poor oral health and cognitive impairment has been reported primarily in older (e.g., age 65+ years) populations (16,20-22). It is not fully established whether this association is present in midlife as well. The association could arise from an effect of impaired cognitive function on oral health or from an effect of poor oral health on cognitive decline. Several mechanisms have been proposed to support both of these possibilities. Older adults with cognitive decline are susceptible to poor oral health since they are unable to perform proper oral care and receive routine dental care less often (19). Moreover, decrease in saliva production, a common side effect of neurological medication, leads to impaired oral clearance and neutralization of dental plaque acid (23). Explanations for the reverse association are that impaired masticatory function resulting from complete tooth loss may influence food choices and affect nutritional status, which in turn, may cause cognitive decline (24). In addition, it has been claimed that periodontal disease can result in an elevation of systemic inflammatory molecules such as serum CRP and can play an

[1]Cognitive function test consists of the Delayed Word Recall (DWR) test, the Digit Symbol Substitution Subtest (DDS) of the Wechsler Memory Scale-Revised, and the first-letter Word Fluency (WF) test.

important role in the pathogenesis of dementia (16,25,26). Lastly, cardiovascular disease and dementia share pathophysiology as well as several common risk factors (10,27,28). Since periodontal disease is a predictor of cardiovascular diseases (29-31), a link between periodontal disease and dementia could be mediated through cardiovascular diseases. However, it is unclear to what extent the association may be accounted for by other factors that contribute to cognitive decline and are associated with poor oral health status or elevated levels of inflammatory markers, such as low education and cardiovascular risk factors. The present study sought to elucidate the associations of poor oral health with developing cognitive impairment by verifying that the association exists in midlife and was not attributable to confounding.

Associations between periodontitis and diabetes have been consistently reported in epidemiologic studies (32-34). Both diseases share a common pathogenesis that involves an increased systemic inflammatory response. In addition, the relationship between diabetes and periodontal disease could be diabetes predisposing to periodontal infection, and once that infection is established, periodontal infection may exacerbate progression of diabetes. A recently ARIC study found that among people with diabetes, periodontal disease was associated with the increased oods for suclinical atherosclerotic heart disease and CHD compared to those with without diabetes or periodontal disease (35). Therefore, we further determined whether diabetes modify the association of periodontal disease with cognitive function.

Elevated levels of inflammatory markers were hypothesized as a mediator of the association between periodontal disease and cognitive function. We justified not adjusting for the mediator to decompose a total effect of periodontal disease on cognitive function into a controlled direct effect (i.e., the effect of periodontal disease on cognitive function not due to inflammation) and an indirect effect (i.e., the effect of periodontal disease on cognitive function that occurs via inflammation) in regression analyses because the adjustment may lead to bias in the estimates. Valid estimation of the direct and indirect effects using the classical approach requires the following assumptions: a)

absence of unit-level exposure-mediator interactions, b) no confounding of the mediator-outcome association, and c) collapsibility of the association measures. In the context of the present study, assumptions a) and b) are major concerns. There must be no individuals for whom periodontal disease status and level of inflammation interact to influence the risk of cognitive decline. This situation is unlikely to occur in real life because the degree of systemic inflammatory marker is a complex interaction of microbiological components and host inflammatory response. If this assumption does not hold, it is impossible to derive a single direct effect of periodontal disease by adjusting for the level of inflammatory biomarkers. Additionally, to adjust for a causal intermediate along with controlling for other confounders of intermediate-outcome association may induce selection biases. Marginal structural models (MSM) fit using inverse probability weights (IPWS) may be an alternative to the classical mediation analysis. MSM can be used to control for confounders and mediators while avoiding selection biases due to conditioning on the causal intermediate (36,37). However, causal explanation of our results was largely constrained by temporality of periodontal disease-inflammatory mediator-cognitive function associations (i.e., no clear evidence of temporal relationships between the exposure, the causal intermediate, and the outcome). For these reasons, only the total effect of periodontal disease on cognitive function was estimated.

Aim 2: Using data from two ARIC sites, estimate associations of oral health measures with eight-year changes (between ARIC Visit 4:1996-1998 and 2004-2006) in cognitive function.

Hypotheses: a) greater number of missing teeth is associated with greater degrees of cognitive decline, and b) severe periodontal disease is associated with greater degrees of cognitive decline.

Overview: The study included ARIC participants who answered dental screening questions at ARIC Visit 4 (1996-1998) and participated the 2004-2006 Brain MRI substudy in two ARIC study sites (Forsyth County NC and Jackson MS). We evaluated associations between oral health

measures and the changes on three cognitive function scores after controlling for socio-demographic factors. For each hypothesis, we also assessed whether combined effects of periodontal disease with diabetes were associated with greater cognitive decline.

Rationale: Periodontal infection in late middle-aged adults may contribute to onset and progression of cognitive decline. However, few longitudinal studies have investigated the association between oral health and cognitive function or dementia (13,20,38,39). It is also not clear whether impaired oral health begins to affect cognitive performance in midlife or if the impact of poor oral health is delayed. The present study investigated the relationship between poor oral health status in late middle-aged adults and changes in cognitive function over the eight years of follow-up. Periodontal infection can possibly manifest as tooth loss (i.e. an ultimate outcome of untreated severe periodontal infection). Therefore, we also studied if tooth loss in late-middle-age adults predicted cognitive decline. Since host defense mechanisms in diabetic individuals have been altered, facilitating bacterial persistence in the periodontal pockets, increasing production of pro-inflammatory cytokines, thus we also examined the potential modifying effect of diabetes on the association between periodontal disease and cognitive decline. With longitudinal study data, standardized measures of periodontal disease, and careful follow-up, we were able to establish clear evidence that poor oral health in midlife predicted changes in cognitive function.

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RESEARCH METHODS

A. Study design

We tested our hypotheses by using ARIC data from Visit 4 (1996-1998) to 2004-2006. ARIC is a prospective, population-based study of vascular diseases in a biracial cohort of middle-aged adults followed from Visit 1 (1987-1989) to Visit 5 (2011-2013). Analyses for each study aim were based on existing data from: (a) main ARIC, (b) Dental ARIC, and/or (c) brain MRI substudy (Figure 3-1). The associations between oral health measures with cognitive function were evaluated after controlling for socio-demographic characteristics and putative confounders. Potential effect modification by diabetes mellitus was also examined.

B. Source of population

At inception (1987-1989), probability samples of men and women, 45 to 64 years of age, were constructed in four different U.S. communities: Forsyth County NC, Jackson MS, suburban Minneapolis MN, and Washington County MD. African Americans were sampled exclusively in Jackson and were oversampled in Forsyth County. The baseline examination included 15,800 men and women, with response rates of 46% in Jackson and 65-67% in the other three communities (1). Follow-up visits occurred every three years through Visit 4, which was completed between 1996 and 1998. Follow-up at Visit 5 will continue through 2013.

C. Data sources and participants' involvement

In the present study, the exposures were obtained from Dental ARIC, a cross-sectional investigation performed at Visit 4 (1996-1998) on a subset of the dentate ARIC cohort, Dental ARIC

consisted of interviews, oral examinations, and collections of GCF, dental plaque and serum. Levels of inflammatory markers related to periodontal disease were identified from GCF and serum samples. A total of 11,656 ARIC participants were seen for Visit 4. Participants were excluded from the Dental ARIC study if they required antibiotics prior to the dental examination (n = 1,621), because pocket depth could not be measured or if they had no natural teeth (n = 1,651). Of the 8,384 individuals eligible to participate in the Dental ARIC study, 6,976 underwent a dental examination (2).

The main outcomes, cognitive test scores, were obtained from ARIC Visit 4 and from the 2004-2006 Brain MRI substudy. Brain MRI studies were carried out among participants from two study sites (Forsyth County NC and Jackson MS) on two separate visits (Visit 3 and 2004-2006) to investigate cerebral abnormalities (e.g., WMHs, SW, and VS). Of the 2,891 participants screened for eligibility during Visit 3, 1,949 participants (67%) received initial brain MRI scan (3). Of those received the initial brain MRI scan, 1,112 participants (57%) underwent a second brain scan and cognitive assessment during 2004-2006 after excluding those who refused and were ineligible (4). Socio-demographic characteristics and related medical histories were abstracted from the ARIC data sets.

Participants were included in the analysis for each study aim if they had received a dental screening or periodontal examination at Visit 4. Data for periodontal status and number of remaining teeth were available only for dentate participants who had an oral examination in Dental ARIC. The dental screening questionnaires were used to identify ARIC participants who were edentulous (complete tooth loss) at Visit 4. Eligible participants were also required to complete cognitive function assessment: a) at Visit 4 for **Aim 1** and b) at the Visit 4 and 2004-2006 for **Aim 2.** (Figure 4-3, 5-7)

D. Sample size and power

Sample sizes that address each aim are shown in Table 3-1. A statistical power analysis was carried out with periodontal status as an exposure (dichotomy) and cognitive function (continuous) as a primary outcome. For aim 1, we used the available sample size for complete case analysis (n ~ 6,400). A two-sided alpha of 0.05 and power of 0.80 were used to calculate minimum-detectable differences in cognitive function at Visit 4, expressed as unit normal deviates for a two-sample t test. Calculated effect sizes represent group differences as the number of standard deviations. Unequal size risk groups were specified (BGI-DL/SB *vs*. others = 1:7). Calculations with the SAS power procedure showed that the minimum detectable mean difference was 0.11 standard deviations (Figure 3-2). By way of comparison, there was a reduction of 0.27 in standard deviations in mean DSS test scores between 1996-1998 and 2004-2006. Therefore, the present study has sufficient power to detect an association of periodontal disease with a difference in cognitive function of a magnitude similar to the declining occurring in the entire cohort with the passage of nine years. For aim 2, the effect of periodontal status on cognitive function were tested using a longitudinal study design in which cognitive scores were repeatedly measured at Visit 4 and 2004-2006. Given the smallest available samples size for complete case analysis (n ~ 500) and an assumed correlation between repeated measures of 0.5-0.6, the present study has 80% power to detect minimal effect size of 0.3-0.4.

E. Assessment of exposures, outcomes, and covariates

Exposure variables

For the present study, the following oral health measures were used as exposure variables indicative of oral disease burden: a) dentate status; b) number of remaining teeth; and c) periodontal status.

Periodontal disease status: Severity of periodontal disease was classified by using BGI, a clinical index reflecting biologic phenotype of periodontal disease based on measures of PPD and

extent of BOP (Table 3-2)(6). Trained examiners (n = 6) recorded periodontal assessments according to Dental ARIC examination protocol. In summary, the periodontal examination included measurements of PPD and gingival recession (GR) at six sites per tooth for all teeth present. PPD was determined with a UNC-15 periodontal probe and recorded in millimeters, with fractions of millimeters rounded to the next lower millimeter. After each quadrant of PPD measures was completed, BOP was assessed and recorded. BOP was determined as either present or absent for each site in a given quadrant. The extent of BOP was expressed as a percentage of all sites. At the same sites, GR was the distance from the cemento-enamel junction (CEJ) to the free gingival margin, and recorded in millimeters with fractions of millimeters rounded to the next lower millimeter. Attachment loss was calculated during data analysis as the sum of PPD and GR. Periodontal examiners at the ARIC centers were calibrated to a standard examiner, and the percent agreement for attachment loss (within 1 mm) between each examiner and the standard examiner, ranged from 83.2% to 90.2%. Weighted Kappa statistics ranged from 0.76 to 0.86, indicating excellent to outstanding agreement (2).

Number of remaining teeth: Examiners recorded the number of permanent teeth including root fragments (range 1-32).

Dentate status: For ARIC participants who received dental screening, we created a binary variable, dentate status (edentulous *vs.* dentate) from two self-reported screening questions: "Do you have any natural teeth?" and "Do you have any dental implants?". People were classified as dentate, if they had any natural teeth. If they had no natural teeth, they were classified as edentulous, even if they had dental implants.

Outcome variables

The main outcome variables for Aims 1 and 2 were continuous measures of three cognitive function tests.

Cognitive function scores: We quantified changes in cognitive function scores measured at the eight-year intervals. Cognitive function assessment consisted of the DWR, DSS, and WF tests. Details of the cognitive assessment protocol have been described elsewhere (3,5). In brief, the DWR is a test of verbal learning and recent memory. Study participants were asked to recall a list of ten common nouns, one at a time. After a five-minute delay, the participant was asked to recall these words by composing a sentence with each one. The DWR score received was the total recalled correctly (score ranges from 0 to 10). The DSS test is a test of concentration and psychomotor speed. Participants were required to translate numbers to symbols using a key within 90 seconds. The total number of correct translations determined the score (a range from 0 to 93). The WF test is a test of expressive language. The participants were asked to generate words beginning with "F", "A", and "S", not including proper names or places, within 60 seconds for each trial. The total score included the combined total of correct words generated for the three trials. Trained examiners administered all assessments in a standardized order during one session in a quiet room. Examiners' performance was monitored by audiotaped recordings. These tapes were reviewed to ensure that the testing procedure was consistent across the study centers.

Covariates

Covariates presumed to modify or confound the associations between oral health and cognitive decline are presented in directed acrylic graphs (DAGs) according to hypothesized causal pathways (Figures 7-1, 7-2). These covariates were and entered as time-independent variables in regression models. Variables at similar levels in the causal paths were grouped to facilitate the identification of causal intermediates and simplify the graphic representation. The following are selected covariates and their coding for the analyses.

Socio-demographic factors were assessed by standard interview at Visit 1. They included gender (male, female), age in years, race (White and African American), education (<12 years, less than high school; 12-16 years, high school completion; or ≥17 years, post-secondary education), and

income (Refused, <$25,000, $25,000-50,000, and >$50,000). A variable representing race and ARIC filed centers was created to control for the racial, regional, and examiner differences in the ARIC cohort as the following: Forsyth/White (FW), Forsyth/Black (FB), Jackson/Black (JB), Minnesota/White (MW), and Washington/White (WW).

APOE genotyping was performed using the TaqMan assay (Applied Biosystems, Foster City, CA). The APOE variants at codons 112 and 158 were detected separately. Data from these two codons were combined to generate the six APOE genotypes as ε2/2, ε2/3, ε3/3, ε4/2, ε4/3, and ε4/4. A binary variable was created to represent the presence or absence of the ε4 allele (5).

Prevalent CHD at Visit 4 was defined as adjudicated myocardial infarction on the electrocardiogram at baseline or prior self-reported history of myocardial infarction, coronary artery bypass surgery, or angioplasty.

Prevalent stroke at Visit 4 was defined as a self-reported history of physician-diagnosed stroke or stroke validated by an ARIC clinician through a review of medical records.

Cardiovascular risk factors at Visit 4 were self-reported hypertension (yes, no), diabetes mellitus (yes, no), hyperlipidemia (yes, no), body mass index (BMI) in kg/m^2, smoking status (never, former, and current), and alcohol use (never, former, and current).

Hypertension was defined as systolic blood pressure ≥ 140 mm Hg, diastolic BP ≥ 90 mm Hg, or hypertensive medication usage in the previous two weeks. Systolic and diastolic blood pressures were measured three times using a random zero sphygmomanometer in the right arm of seated participants; the mean of the last two measurements was calculated for each individual.

Diabetes mellitus was defined as a fasting glucose level of ≥126 mg/dL, nonfasting glucose ≥ 200 mg/dL, self-reported history of diabetes, or regular pharmaceutical treatment for diabetes. Participants were asked to fast for 12 hours before the clinic visit. Blood was drawn from the antecubital vein of seated participants, and serum glucose was assessed by the hexokinase method.

Hyperlipidemia was defined as LDL cholesterol of ≥140 mg/dL or the use of cholesterol-lowering agents. Plasma lipids and lipoproteins were determined by enzymatic methods in a laboratory standardized by the CDC. LDL cholesterol was estimated by the Friedewald equation[1].

BMI was calculated as weight in kilograms divided by the square of height in meters. A continuous measure of BMI will be used for the analyses.

Smoking was self-reported as never smoked, former smoker, or current smoker.

Alcohol drinking was assessed from subject self-report and described as never, former, or current drinker.

F. Statistical analyses

Overview of analytical approach

All analyses were performed using SAS version 9.3 (SAS Institute, Inc., Cary, North Carolina). We primarily used a complete case analysis for the outcome variables, and assessed frequency and pattern of missing independent variables. Candidate variables were eliminated if their distributions were too narrow to be meaningfully predictive or they had a substantial proportion of missing values (> 20%). Initial descriptive statistics and plots were generated. Means and percentages for each outcome variable were assessed in bivariate and stratified analyses.

Study hypotheses were evaluated using generalized linear models (GLMs) fit with the SAS PROC GENMOD procedure to examine associations between oral health measures and cognitive function. GLMs are flexible and well suited to a wide range of situations, including traditional general linear regression. For a normally distributed continuous variable (e.g., cognitive test scores), we used least-squares linear regression, for which the "identity" link function was specified. In addition, we used an extension of GLMs, marginal generalized estimating equations (GEE) models, for a repeated

[1]Friedewald formula: LDL = TC – HDL- TG/0.5 (mg/dL)
TC, Total cholesterol (mg/dL); HDL, High-density lipoprotein (mg/dL); TG, Triglycerides (mg/dL)

outcome (cognitive function scores), where a within-subjects correlation was taken into account. In this case, the GENMOD procedure offers a "REPEATED" statement to analyze such correlated outcomes data.

DAGs and the change-in-estimate procedure were used for adjustment-variable selection. We selected explanatory variables based on published studies and biological plausibility of the relationships. DAGs showed the selected covariates and their relationships with the exposures and outcomes in the causal paths. We also used results of bivariate, stratified, and collinearity analyses to guide the selection of variables for model building.

Descriptive statistics

Descriptive analyses were initially performed on selected variables to study data distributions as well as identify missing values, impossible values, and outliers for outcomes, exposures, and covariates. We used PROC UNIVARIATE to examine mean, median, mode, standard deviation, range, skewness, and kurtosis for each continuous variable (e.g., cognitive score, number of teeth, and levels of inflammatory markers). In addition, data distributions were visualized using graphical displays such as box plots and scatterplots. Frequency distributions were evaluated using PROC FREQ for ordinal and categorical variables (e.g., periodontal disease status, CHD, and stroke). The number, percentage, and type of missing data were recorded for each variable.

Bivariate and multivariate analyses

We conducted bivariate analyses to investigate the nature and strength of the following associations: (a) between explanatory variables and both outcomes, and (b) among explanatory variables. These analyses helped to identify potential cofounders, effect modifiers, and collinearity for regression model building. First, we calculated a crude estimate of the association between the exposure and the outcome using general linear regression for continuous outcomes (mean ± SE).

Second, we examined stratum-specific estimates for the presumed effect modifier (diabetes mellitus). If the stratified estimates were substantively different from each other and a likelihood ratio test for homogeneity suggests strong effect modification ($p < 0.10$), then we included an interaction term between the effect modifier and the exposure in the regression model. Next, we determined whether a covariate was a potential confounder by assessing a magnitude and the 95% confidence interval along with the p-value between the covariate and the exposure or outcome. Then, we compared magnitude and precision of the estimates for the exposure-outcome association with and without adjustments for that covariate. If the estimate adjusted for a selected variable differed from the crude estimate by more than 10%[2], we considered that covariate to be a potential confounder.

Collinearity and multicollinearity among continuous and ordinal explanatory variables were investigated using PROC REG with options VIF, TOL, and COLLIN in SAS. For nominal or categorical independent variables, the same approach was applied. We created dummy variables for each category and use our outcome of interest as the dependent variable in the linear regression. We examined tolerance[3] and variance inflation factor (VIF) for each variable. The VIF is 1/Tolerance; it is the number of times the variance of the corresponding parameter estimate is increased due to multicollinearity as compared to as it would be if there is no mulitcollinearity. Values of VIF exceeding 10 suggest multicollinearity. If the variables were correlated covariates, coding choices were reconsidered or one variable may be dropped from the analyses.

Analysis plan for specific aim 1

We estimated associations of the following oral health measures with cognitive function:

a. Dental status

b. Tooth loss

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[2]The change in estimate is the absolute difference between the crude estimate and the adjusted estimate divided by the adjusted estimate: $(\beta_{crude} - \beta_{adjusted}) \times 100$. A priori criterion for a potential confounder is that the change in estimate is greater than 10%.

[3] For each independent variable, tolerance = 1-Rsq, where Rsq is the coefficient of determination for the regression of that variable on all remaining independent variables, low values indicate high multivariate correlation.

c. Periodontal disease classified by BGI

The conceptual basis for these analyses was that poor oral health can be predictive of low cognitive performance. The main hypothesis was that persons who are edentulous, have lost most of natural teeth, or have severe periodontal disease have lower cognitive scores. We also investigated whether diabetes mellitus was an important modifier of the relationship between each predictor and cognitive function. Separate regression models were applied for each outcome-exposure association. Independent variables included an ordinal measure of periodontal disease (five levels forBGI index), continuous measures of remaining teeth, and a binary measure of dental status (edentulous or dentate). Dependent variables were continuous measures of three cognitive functions. Thus, linear regression was used to analyze the data. The linear trend across the periodontal status categories was tested. First, we evaluated the primary effect for each main outcome, adjusting for age, gender, race-center, education, and income. Second, we developed a fully adjusted model by including all potential confounders and effect measure modifiers based on DAGs and bivariate analyses. A reduced model was also created using the minimally sufficient set of covariates for adjustment used in the multiple analyses included age, race, gender, study sites, education, income, smoking, alcohol use, and diabetes. Covariates in fully adjusted models consisted of variables from the reduced model and variables that were significantly associated with exposures or outcomes, namely body mass index (BMI), hyperlipidemia, hypertension, and APOE ε4. Significance of effect modification by diabetes was assessed with likelihood ratio tests by comparing the -2log-likelihood of the fully adjusted model (i.e. model included the interaction term between the exposure and diabetes) with that of the nested model (i.e. model excluded the interaction term). The interaction term was removed if its p-value was greater than 0.10. Third, the change-in-estimate approach was used to select the final model. Regression coefficients for oral health measures (i.e., dental status, the number of teeth, and BGI) from the reduced model and those from the fully adjusted model were compared. If the change-in-estimate was less than 10% or ± 0.1, the more parsimonious model or the reduced model was selected.

Analysis plan for specific aim 2

We used data from two ARIC sites to estimate associations of oral health status measures (e.g.,BGI index, tooth loss, and complete tooth loss) with the eight-year change in cognitive function between ARIC Visit 4:1996-1998 ("baseline") and 2004-2006 ("follow-up"). A longitudinal analysis using the GEE method was applied for the cognitive function measures. The dependent variables were continuous measures of three cognitive function scores (DSS, DWR, and WF). We created an indicator variable, namely time (*t*), to identify whether the scores represented a baseline or follow-up measurement. We assumed unstructured correlation for all pairs of within-subject outcomes (i.e., cognitive score); therefore, working correlation matrix as "unstructured" was specified for the correlated responses in the analyses. In the model, the main effect of time indicated if the cognitive scores significantly changed over the 8-year follow-up. The interaction term between time and the exposure was added to determine whether there was a significant difference in the change of cognitive scores between participants who had poor oral health and those who did not.

$$E\left(Y_{it}\middle|X_{it}\right) = \beta_0 \pm \beta_1 X \pm \beta_2 t \pm \beta_3 X * t \pm \sum_{m=1}^{M} \beta_{4m} G_{im} \pm \varepsilon_{it}$$

Above is the fully adjusted model where Y_{it} is an observation for subject i at time t; β_0 is the intercept; x is the oral health measure; β_1 is the regression coefficient for the oral health measure x; β_2 is the regression coefficient for time; $x * t$ is an interaction term between Visit 4 oral health measure and time; β_3 is the regression coefficient for the interaction term; G_{im} are the values of the m time-independent covariates for subject i; β_{4m} are the regression coefficients for the time-independent covariates; M is the number of time-independent covariates; and ε_{it} is the error for subject i at time t.

Since GEE fits models using a quasi-likelihood method, not a maximum likelihood, "Quasi-likelihood under the independence model information criterion" (QIC) was used to assess the model fit. A smaller QIC suggests a better fit of the model.

G. Protection of human subjects

Human subjects involvement, characteristics, and study design

The present study was a secondary analysis of the ARIC, a prospective, population-based study of vascular diseases. The final datasets for the analyses were derived from three main measurements: a) cognitive function assessments; b) Brain MRI; and c) dental screening and examination at ARIC Visit 4. Our primary hypotheses were that: a) poor oral health was predictive of early cognitive decline and rapid progression of the decline over time; and b) the magnitude of change in cognitive test scores was greater among individuals with few teeth or an extensive form of periodontal disease.

Participants were from four geographic areas in the US, which were Forsyth County NC, Jackson MS, the suburbs of Minneapolis MN, and Washington County MD. We used ARIC data from Visit 4 through 2004-2006. To address each study aim, eligible study subjects for the analyses were ARIC participants who underwent a dental examination or screening and cognitive or Brain MRI assessment. Estimated samples sizes are presented in Table 3-1.

Sources of materials

Statistical analyses were based on data available from the ARIC Coordinating Center at UNC-Chapel Hill. Information obtained from participants included socio-demographic status (age, gender, race, study site, education, and marital status), cognitive performance (DWR, DSS, and WF), oral health measures (dentate status, number of teeth, and periodontal disease), cardiovascular risk factors (hypertension, diabetes, smoking status, alcohol use, etc.), CHD, stroke, and APOE genotype. Strict data confidentiality procedures have been established for the ARIC study. They are applicable

to all ancillary studies and are followed here. Only the ARIC study principal investigators have access to individually identifiable information of subjects. The principal investigator for the present study received de-identified files from the ARIC Coordinating Center for data analyses.

Potential risks and adequacy of protection against risks

This study is based on an existing dataset, and there is no direct interaction with subjects. Therefore, risk of physical harm to subjects is not applicable and potential for psychological harm limited to breach of confidentiality. After merging data from all study visits, personal identifiers was removed from the dataset for each visit by ARIC Coordinating Center staff. The final dataset is a de-identified dataset and, thus, the risk of disclosure is minimal. For security purposes, the final dataset is stored in the School of Dentistry research database, which is password enabled and backed up periodically. Only authorized personnel have access to the dataset, and the password is changed periodically.

Potential benefits of the proposed research to human subjects and others

Since the study involved no direct interaction with study participants, there was no direct benefit to the individual subjects. However, results from the study can provide important medical information to society as a whole. The study allowed us to assess whether tooth loss and periodontitis, based on clinical measurements of periodontal status are independently associated with cognitive decline in later life. If the association exists, periodontal treatment or prevention may be a promising strategy to reduce the incidence of cognitive impairment and dementia.

Importance of knowledge to be gained

The costs and social burden resulting from cognitive impairment and dementia are increasing in the U.S. and worldwide. This project contributes novel scientific knowledge that addresses a major public health concern in older adults. To our knowledge, this is the first study to examine associations between periodontal disease (BGI index) and cognitive function using prospective longitudinal data.

H. Tables

Table 3-1. Numbers of subjects participating in the ARIC study from Visit 1 through Visit 4, Dental ARIC, and Brain MRI

Study/Examination		1987-1989 Visit 1	1990-1992 Visit 2	1993-1995 Visit 3	1996-1998 Visit 4	2004-2006
Main ARIC		15,800	14,348	12,887	11,656	
Cognitive function			14,201	2,077	11,343	1,112
ARIC Brain MRI				1,949		1,112
Dental ARIC: Dental screening					11,378	
Dental ARIC: Dental examination					6,967	
Proposed study: Expected number of participants for each study aim and exposure						
Aim 1	(a) Dental status				~10,000	
	(b) Tooth loss				~6,400	
	(c) PD				~6,400	
Aim 2	(a) Dental status			~900		
	(b) Tooth loss			~500		
	(c) PD			~500		

Table 3-2. Definition and prevalence of periodontal disease classified by Biofilm-Gingival Interface (BGI) system in adults aged 52 to 74 years at ARIC Visit 4

Periodontitis Classification (6)	Definition	Prevalence
No pockets/ Low bleeding (BGI-H)	All PPD ≤ 3 mm, BOP < 10%	14.3%
No pockets/ Moderate-Severe bleeding (BGI-G)	All PPD ≤ 3 mm, BOP ≥ 10%	15.1%
Pockets/Low bleeding (BGI-DL/LB)	One or more PPD ≥ 4 mm, BOP < 10%	18.0%
Pockets/Moderate bleeding (BGI-DL/MB)	One or more PPD ≥ 4 mm,10% ≤ BOP < 50%	39.7%
Pockets/Severe bleeding (BGI-DL/SB)	One or more PPD ≥ 4 mm, BOP ≥ 50%	12.9%

PPD, periodontal probing depth; BOP, bleeding on probing
Offenbacher S, Barrons SP, Singer RE, *et.al.* Periodontal Disease at the Biofilm-Gingival Interface. J Periodontal 2007;78;1911-1925.(6)

Table 3-3. Summary of candidate variables, coding, and analytic approach

Variables	Coding	Aim 1	Aim 2
Visit 4 DWR, DSS, WF	Continuous	Outcome	Outcome
2004-2006 DWR, DSS, WF	Continuous	Not used	Outcome
BGI	Categorical	Exposure	Exposure
Number of teeth	Continuous	Exposure	Exposure
Dentate status (Yes / No)	Binary	Exposure	Exposure
Stroke (Yes / No)	Binary	Covariate	Covariate
CHD (Yes/ No)	Binary	Covariate	Covariate
Age	Continuous	Covariate	Covariate
Gender (Female / Male)	Binary	Covariate	Covariate
Education (<12, 12-16, ≥ 17 years)	Categorical	Covariate	Covariate
Income (< $25,000, $25-50,000, >$50,000, refused)	Categorical	Covariate	Covariate
Race-center (FB, FW, MW, WW, JB)	Categorical	Covariate	Covariate
APOE ε4 genotype (Yes / No)	Binary	Covariate	Covariate
BMI	Continuous	Covariate	Covariate
Hyperlipidemia (Yes / No)	Binary	Covariate	Covariate
Hypertension (Yes / No)	Binary	Covariate	Covariate
Diabetes mellitus (Yes / No)	Binary	Covariate	Covariate
Smoking (Current/ Former/Never)	Categorical	Covariate	Covariate
Alcohol (Current/ Former/Never)	Categorical	Covariate	Covariate
Time (Baseline/Follow-up)	Binary	not used	Covariate
Interaction terms			
BGI x Diabetes		Yes	Yes
(BGI, number of teeth, dentate status) x Time		Not used	Yes
Models			
General linear regression		Yes	No
GEE		No	Yes

I. Figures

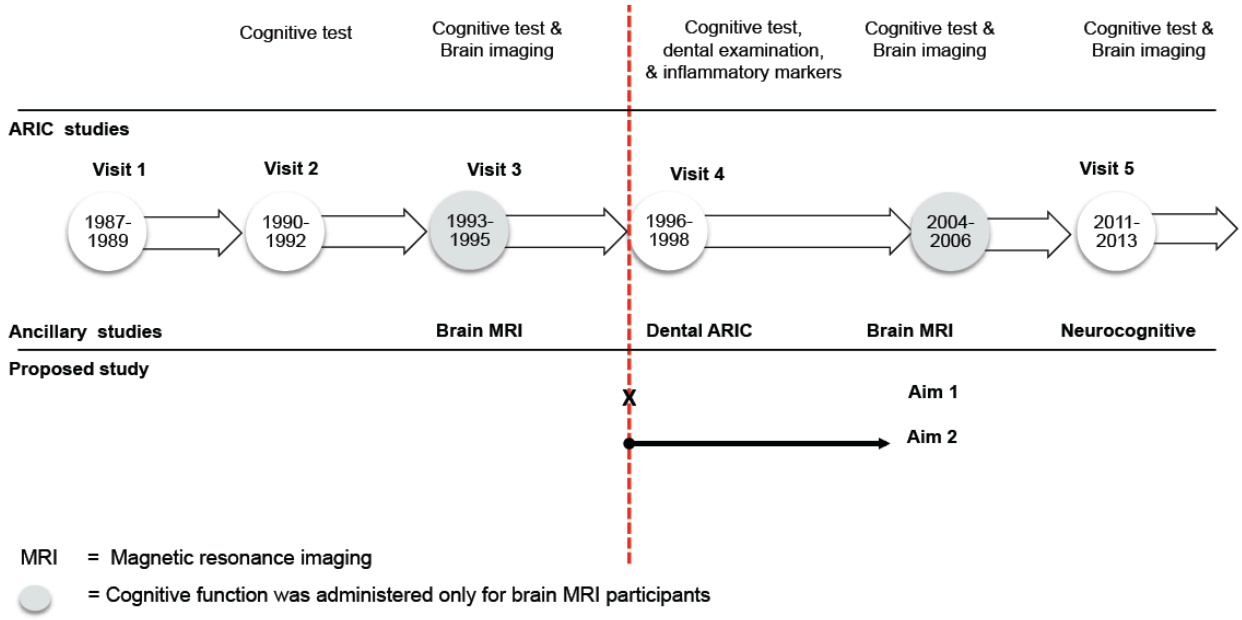

Figure 3-1. Data sources, key variables, and study design for each specific aim

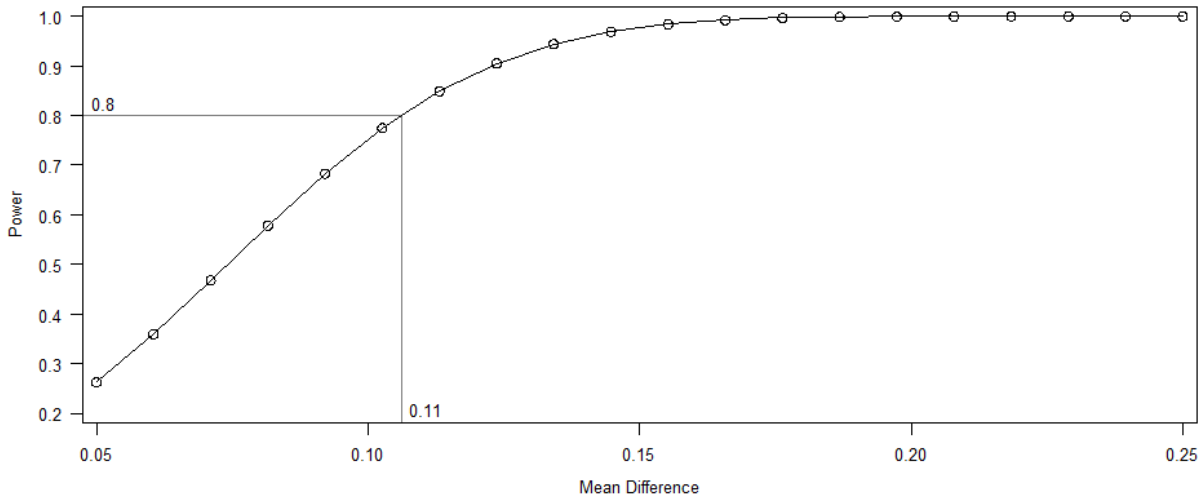

Figure 3-2. Power plot for detectable difference in means between BGI-DL/SB *vs.* others (Difference is expressed as a number of standard deviations.)

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STUDY 1: RESULTS

Associations between oral health measures and cognitive function in late middle-aged adults: A community-based study

A. Overview

Associations between tooth loss, periodontal disease, and cognitive function have been reported primarily in older adults. It has not been established whether this association is present in late middle-aged adults as well. The purpose of this investigation was to determine whether tooth loss and periodontal disease were associated with lower cognitive function in adults aged 52-75 years who participated in the Atherosclerosis Risk in Communities (ARIC) study. Cognitive function was assessed in 11,097 participants between 1996 and 1998 (Visit 4) by three cognitive tests: Delayed Word Recall (DWR), Digit Symbol Substitution (DSS), and Word Fluency (WF). At Visit 4, 9,874 answered dental screening questions, and 5,942 of the 8,554 dentate participants received comprehensive oral examinations, including periodontal probing. Multiple linear regression models used dental status, number of teeth, or clinical periodontal conditions classified by the Biofilm-Gingival Interface (BGI) index to predict cognitive scores, adjusting for socio-demographic factors, smoking, alcohol use, and diabetes. Approximately 13 % of participants were edentulous. 27.3% of dentate participants had < 20 teeth and 12.4 % had pocket depth ≥ 4 mm with severe bleeding. Compared to dentate participants, edentulous subjects had lower scores for all cognitive tests. Among the dentate, fewer teeth and gingival bleeding were associated with lower DSS and WF scores, although periodontal pocket depth was not. Complete tooth loss and gingival bleeding were markers of poorer cognitive function. These findings indicate that association between periodontal

inflammation and cognition that has been observed in older adults is present in late middle-aged adults. The association with tooth loss suggests that oral disease earlier in life might be a marker of future cognitive decline in late middle-age. The association with gingival inflammation illustrates the potential for bi-directional relationships between oral disease and cognitive decline.

B. Introduction

As the populations age, cognitive impairment and dementia are becoming challenging public health problems because they adversely affect older adults' quality of life and health care costs (1). Many studies, primarily in older adults have linked oral diseases and/or previous tooth loss with low cognitive performance and dementia onset (2-10). However, although the associations are clear, they could arise from various mechanisms. For example, persons with cognitive impairment have reduced ability for oral hygiene, which would lead to poor oral health (11). Cross-sectional studies, which are typically unable to establish the temporal sequence underlying causal associations, have provided most of the evidence to date.

In addition, if the mechanism behind the association begins with cognitive impairment, an extended interval would be needed for the subsequent decline in oral health to translate into tooth loss and edentulism, making that scenario less probable. Therefore, a more likely reason for the association may be that poor oral health leads to cognitive decline. Several studies suggest that periodontal infection and increased inflammatory levels in early life could be responsible for later cognitive decline (6,7,12-14). For example, a case-control study found oral bacteria more frequently in the brains of Alzheimer's disease (AD) than non-AD subjects (15). A recent pilot study using high throughput DNA sequencing reported higher levels of *Prevotellaceae* from subgingival plaque in participants with dementia (16). It is also possible that periodontal disease may affect cognition in midlife (17).

Many studies of the association between cognitive performance and periodontal disease have used a wide range of indicators of past and current periodontal disease, including tooth loss (3,5,6,8,18), presence of microorganisms (15), immunological biomarkers (13,14,16,19), and clinical signs of periodontal destruction and inflammation (3,5,7,8,12). Tooth loss is an imperfect indicator of periodontal disease since tooth loss can result from non-inflammatory causes (e.g., caries and trauma). More direct evidence comes from studies using oral bacteria burdens and systemic antibody levels, which strongly suggest that periodontal disease contributes to the pathogenesis of neurodegenerative diseases and cognitive decline. Although most of these studies are small, high levels of immunoglobulin G antibody against the periodontal pathogen *P. gingivalis* were found to be associated with cognitive impairment in a large U.S. national health survey, NHANES (13). In addition, a recent longitudinal study, with an average follow-up of 10 years, showed that individuals who developed Alzheimer's disease (AD) had higher baseline levels of serum antibodies to *F. nucleatum* and *P. intermedia* (14).

These two studies provide important evidence, but they examined only two of the dozens of known periodontal pathogens. Studies that have investigated associations between cognitive decline and clinical signs of periodontal disease (e.g., pocket depth, gingival bleeding, or attachment loss) have reported inconsistent results (2,5,7,8,12,17,19). Possible reasons are that many of these studies are small (7,12,19) and have relied on measures of periodontal disease based on partial examinations (8,17) or self-report (2). In addition, attachment loss, often used as an indicator of past periodontal disease, may also reflect non-inflammatory processes such as gingival recession from physical injury from tooth brushing or flossing.

The purpose of the present study, therefore, was to examine associations between cognitive performance in midlife adults and periodontal disease assessed with an index that reflects current state of probing depth and inflammation. Toward that end we related three measures of cognitive performance to three indicators of oral health – dental status, number of teeth, and periodontal disease

classified by the Biofilm-Gingival Interface (BGI) classification–among middle-aged adults in the Atherosclerosis Risk in Communities (ARIC) study. We hypothesized that lower cognitive performance was associated with edentulism, tooth loss, and severity of periodontal disease.

C. Methods

Design and study population

The present cross-sectional study used ARIC and Dental ARIC data collected between 1996 and 1998 (Visit 4). Details regarding the study designs and protocols have been reported elsewhere (20-22). Briefly, ARIC is a prospective, community-based study of middle-aged adults (age 45-64 years at inception) followed since 1987-1989 (Visit 1). Dental ARIC, an ancillary study of the parent ARIC study, was conducted at Visit 4. We confined our analysis to African American and white participants who received a cognitive assessment and dental screening interview at that visit. A subset of screened dentate participants also underwent comprehensive dental examination, which included periodontal probing. Of 11,097 participants who completed Visit 4 cognitive battery tests, 9,874 answered dental screening interviews that asked about tooth loss. Of the 8,554 dentate respondents, 5,942 received a dental examination (Supplemental Figure 4-3).

Oral health measures

Exposures included dental status, number of teeth, and clinical classification of periodontal disease. An individual's dental status was obtained from answers to the following two items on a self-administered questionnaire: "Do you have any natural teeth?" and "Do you have any dental implants?" Participants with only dental implants (n = 21) were excluded from the study. During the dental examination, the number of teeth present was recorded. Periodontal probing depth (PPD) and bleeding on probing (BOP) were assessed at six sites on all remaining teeth by trained examiners.

The BGI system has been developed based on a concept that clinical disease classification should reflect an underlying biological process of periodontal disease that involves a complex

interaction of the microorganisms with host inflammatory and immune response.BGI index, based on measures of PPD (≤ 3 mm or ≥ 4 mm) and the extent of BOP (<10%, low; 10-50%, moderate; or ≥ 50 %, severe), was used to classify periodontal status into five levels. Subjects with PPD ≤ 3 mm (no periodontal pockets) were defined as periodontal healthy if BOP was less than 10% or gingivitis if BOP was 10% or more. Subjects with one or more PPD ≥ 4 mm (had periodontal pockets) were divided into low, moderate bleeding, or bleeding. During the dental examination, the number of teeth present was recorded (23).

Cognitive function

Outcomes of interest were scores from the following cognitive tests: a) Delayed Word Recall (DWR); b) Digit Symbol Substitution (DSS); and c) Word Fluency (WF). The DWR tests verbal learning and recent memory. The WF, a test of expressive language, and the DSS, a test of concentration and psychomotor speed, assess executive function. Higher scores on each of the three tests indicate better cognitive ability. All cognitive tests were administered by trained examiners. Cognitive test protocols from ARIC have been reported elsewhere (21).

Covariates

Covariates included socio-demographic factors (age, race, gender, educational level, income, and study sites), cardiovascular risk factors, APOE genotype, stroke, and coronary heart disease (CHD). Educational levels were classified as less than high school, high school completion, and post-secondary education. Household income was coded as <$25,000, $25,000-$50,000, > $50,000, and refused (1996-1998 dollars). The four ARIC communities are Forsyth County, NC; Jackson, MS; the northwest suburbs of Minneapolis, MN, and Washington County, MD. A variable representing race and ARIC filed centers was created to control for the racial, regional, and examiner differences in the ARIC cohort as the following: Forsyth/White, Forsyth/Black, Jackson/Black, Minnesota/White, and Washington/White. Cardiovascular risk factors included smoking and alcohol use (each recorded as never, former, or current), diabetes, hypertension, hyperlipidemia, and body mass index.

Apolipoprotein E (APOE) genotype was dichotomized as presence or absence of the APOE ε4 allele (Supplemental methods).

Statistical analyses

Gender- and race-specific distributions of covariates and self-reported dental history were examined. Bivariate analyses were used to assess associations of covariates with cognitive scores and oral health measures. Cognitive scores were analyzed as continuous measures using multiple linear regression models. We used directed acyclic graphs (DAGs) and the change-in-estimate procedures to select the adjustment variables in this study. CHD and stroke were considered mediators of the exposure-outcome association and were therefore not included in the regression models. Models fit with a minimally sufficient set of covariates, identified in the DAGs, included age, race, gender, study sites, education, income, smoking, alcohol use, and diabetes (DAG models). Fully adjusted models included these variables along with body mass index (BMI), hyperlipidemia, hypertension, and APOE ε4. The regression coefficient for the oral health measure (i.e., dental status, number of teeth, or BGI) from the DAG model was compared with that from the fully adjusted model. If the estimate changed by less than 10 % or ± 0.1, the more parsimonious (DAG) model was selected for the primary analysis. Supplementary analyses used other clinical measures of periodontal disease including the extent of attachment loss and CDC/AAP classification as predictor variables. The effect of BGI components (PPD and BOP) on cognitive scores was also determined separately. The statistical package SAS version 9.3 (SAS Institute, Cary NC) was used for all analyses.

D. Results

Characteristics of study participants

The majority of the study sample was female (56 %). About 80% of all participants were whites, but all Jackson participants and about 9 % Of Forsyth participants were African Americans. About half of the participants were current drinkers, and 14% were current smokers. Compared to

white participants, African American subjects had less education, lower incomes, more medical problems, and lower scores on all cognitive tests. Of 9,874 dentally screened participants, 13.4 % (1,320) were edentulous. More than two-thirds of participants who received a comprehensive dental exam had periodontal pockets (PPD ≥ 4 mm), and 12% were classified as periodontitis with severe bleeding (Table 4-1). About 70% of edentulous and dentate participants reported past tooth loss due to caries. Edentulous participants were two times higher (32 % *vs*. 11%) than dentate participants to report past tooth loss due to "gum disease," and 42% of edentulous participants had an initial "gum disease" diagnosis more than 30 years ago (Table 4-2).

In the multiple regression analyses, hypertension, body mass index, hyperlipidemia, and APOE ε4 were not confounders for any of the associations between oral health measures and cognitive scores. Therefore, results from the DAG models are presented in Table 4-3.

Tooth loss and cognitive function

Complete tooth loss was significantly associated with lower DWR, DSS, and WF scores (Table 4-3, Figure 4-1). Regression coefficients for complete tooth loss were greatly attenuated after adjusting for socio-demographic factors (unadjusted associations are shown in Supplemental Table 4-5). The associations remained significant after controlling for smoking, alcohol, and diabetes. Among the dentate participants, a larger number of teeth present were associated with higher cognitive scores for all tests. However, the number of teeth was no longer associated with DWR scores after adjusting for socio-demographic factors. The adjusted associations with DSS (b = 0.069 per tooth, *p-value = 0.0003*) and WF (b= 0.087, *p-value 0.0002*) were significant in the final models (Table 4-3).

Periodontal disease and cognitive function

In the crude analyses, periodontal disease was associated with all cognitive test results, and the severe periodontitis groups had the lowest cognitive scores (Table 4-5). As with the analysis for number of teeth, the association with DWR scores was not evident after adjustment for socio-

demographic factors. In the final models, BGI was significantly associated with DSS (*p-value = 0.0464*) and WF (*p-value = 0.0015*); however, there was no clear dose-response relationship across the five levels of the BGI index. The lowest cognitive scores were found in participants with gingivitis or periodontitis with severe bleeding (Table 4-3, Figure 4-2).

Supplementary analyses showed that BGI index was correlated with the extent of attachment loss. In other word, periodontitis with severe bleeding group had the greatest extent of attachment loss (Table 4-7, Figure 4-5). For each of the three cognitive tests, low scores were correlated with greater plaque deposit and attachment loss, but not with CDC/AAP classification and pocket depth (Table 4-8).

E. Discussion

In this late middle-aged cohort, participants with no teeth had lower cognitive scores on all three tests. The other two indicators of poor oral health—decreases in number of teeth and periodontal disease—were associated with lower DSS and WF scores, but not with lower DWR scores. We did not observe a dose-response relationship between five levels of periodontal disease created by the combination of PPD and BOP with cognitive performance. Instead, cognitive scores were likely to be related to the extent of BOP.

Strengths of this study include its large population-based sample and high quality control for periodontal and cognitive function assessments. Clinical examination for periodontal disease, coded based on biological systems at the biofilm-gingival interface, (23) allowed us to investigate the association between cognitive function and degree of periodontal infection and inflammation. A fundamental limitation of our study is that our data are cross-sectional data, so associations could reflect effects of poor oral health on low cognitive performance or the reverse process. Furthermore, the Dental ARIC study enrolled only people with no contraindication to periodontal probing (i.e., a requirement for antibiotic prophylaxis). If people who require antibiotic prophylaxis have medical

conditions that are associated with severe periodontal disease, this exclusion could cause the association with cognitive function to be underestimated. Another limitation is that our cognitive tests cover only two cognitive domains, memory (DWR) and executive function (DSS and WF).

Although tooth loss and cognitive function were measured cross-sectionally, multiple tooth loss in this study sample typically occurred early in life, likely before the beginning of age-related cognitive decline (Table 4-2). Thus, the association between edentulism and lower cognitive performance seems more likely to reflect an effect of past periodontal disease on cognitive function rather than the development of inflammation from current low cognitive performance. On the other hand, at least part of the positive association between tooth loss and cognitive function may be attributable to socio-demographic factors. Having no teeth remained a significant term in the multiple regression model for low cognitive function that included terms for health behaviors and medical conditions, but residual confounding by socioeconomic status is a distinct possibility. By contrast, the authors of a large cross-sectional study which included middle-aged adults (45 years and older) suggested that an association they observed between tooth loss and word list recall test scores was likely due to confounding because it disappeared after they controlled for socioeconomic status (24).

Our results are consistent with those from studies that suggest that loss of multiple teeth early in life increases the risk of cognitive decline (2,3,7,10). For instance, a case-control study in monozygotic twins reported that only history of tooth loss before age 35 years was a significant risk factor for AD (10). A cross-sectional study in Japanese older adults reported an association between having an extended edentulous period (15 years or longer) and increased risk of low cognitive scores (3). However, both of these studies were retrospective and relied on self-reports of tooth loss.

Two possible biological pathways whereby tooth loss could accelerate cognitive decline have been proposed: a) systemic infection and resultant inflammation (7,25); and b) nutritional deficiency (4,26). Even though we did not measure nutritional status, that mechanism is unlikely to explain the

association between tooth loss and low cognitive performance in our study. In the study of older Japanese adults having few teeth without dentures increased the risk of dementia onset (Hazard ratio = 1.85; 95% CI 1.04, 3.31) (4). But virtually all ARIC edentulous participants (98%) had dentures and also tended to have BMIs higher than those for dentate participants (data not shown).

Among the dentate participants in our study, both number of teeth and periodontal disease were associated with DSS and WF scores, but these associations were relatively weak. Examination of cognitive scores in relation to the levels of the BGI index indicated that lower cognitive function was related primarily to the extent of gingival bleeding rather than to periodontal pocket depth. Our finding was consistent with a previous cross-sectional study analyzing the NHANES data, where the extent of gingival bleeding was associated with low scores on two cognitive tests (i.e., symbol digit substitution and serial digit learning test) in young and middle-aged adults (20-59 years)(17).

The BGI index reflects both the current and the cumulative burden of inflammatory periodontal disease. Both gingivitis and deep pockets were associated with lower cognitive scores, suggesting two causal scenarios. In the first scenario, deep pockets are a marker of chronic inflammatory periodontal disease which is a cause of cognitive decline. In this scenario, gingivitis represents a marker of current inflammation and periodontal pockets represent a marker of previous inflammation. Participants with both gingivitis and deep pockets therefore are expected to have the lowest cognitive scores because of longer period of infection and elevated inflammatory levels. However, the effect of periodontal pockets probably is underestimated in this scenario because teeth with very deep pockets are likely to be extracted, thereby underestimating the estimated effect of periodontal pockets. . Likewise, periodontal treatment is likely to reduce pocket depth, but it will not reduce inflammation unless oral hygiene or host-response to inflammation also improves. Hence, the gingivitis group could represent people whose periodontal disease has been treated. In our study sample, about half of dentate participants reported less than 10 years of having “gum disease” and about 11 % have lost teeth because of “gum disease” (Table 4-2). A longitudinal study in older men

found that additional tooth loss with progression of alveolar bone loss or progression of pocket depth per decade predicted low cognitive function after a 32-year follow-up interval (5).

In the second scenario, a reverse causal process may be possible. Gingivitis represents the consequences of poor oral hygiene, which itself is a consequence of cognitive decline. Specifically, an early decline in executive function domain (DSS and WF tests) may alter individuals' cognitive processes (e.g. planning, reasoning, initiation, or making decisions) and perception toward health care, resulting in non-compliance with oral hygiene behavior. Our previous study (27) revealed that six-year changes (between 1990-1992 and 1996-1998) in executive function were associated with infrequent tooth brushing, plaque deposit, and Löe and Silness gingival index.

The clinical method used to measure gingivitis casts some doubt on the second scenario. Unlike Löe and Silness gingival index, BOP detects bleeding anywhere in the pockets, including ulceration of deepest tissue in the pocket. In principle, we expect that BOP in sites with deep pockets is influenced less by supragingival plaque deposits than BOP in shallow lesions or gingivitis group. However, counter to this expectation, Offenbacher et.al (2007) (23) reported higher prevalence of infrequent tooth brushing in severe periodontitis than that of gingivitis groups. Given discrepancies between these expectations and the observed relationships, and given the cross-sectional nature of study, it is difficult to evaluate the merits of these two scenarios.

Further relevance and interpretation of supplementary analyses were discussed in Chapter 6.

F. Conclusion

Our study findings add to the evidence that complete tooth loss, low number of teeth, and the inflammatory stage of periodontal disease are associated with lower cognitive performance. Because in our study severe periodontal disease and edentulism apparently occurred many years before data collection, we believe that the process through which these conditions developed preceded rather than

followed age-related cognitive decline. However, the association of poorer cognitive function with gingivitis could reflect an effect of cognitive decline, so the association between oral health and cognitive function could reflect causal processes in either – or both – directions.

G. Human participants protection

No protocol approval was necessary because this study involved the analysis of secondary data only.

H. Tables

Table 4-1. Race- and gender- specific of ARIC Visit 4 characteristics of study samples

Characteristics	African American		White		
	Female (n = 1,301)	Male (n = 693)	Female (n = 4,209)	Male (n = 3,671)	All (n = 9,874)
Age at Visit 4, mean ± SD	61.6 ± 5.6	61.7 ± 5.8	62.7 ± 5.6	63.5 ± 5.6	62.8 ± 5.7
Study sites, %					
Forsyth	11.7	12.1	28.8	28.7	25.4
Jackson	88.3	87.9	0	0	17.8
Minneapolis	0	0	36.1	37.0	29.1
Washington	0	0	35.1	34.3	27.7
Education, %					
Less than high school	32.4	32.0	13.4	14.6	17.6
High school completion	31.6	27.9	51.3	39.5	42.7
Post-secondary education	36.0	40.1	35.3	45.9	39.7
Income, %					
Refused	2.5	2.7	2.1	2.1	2.2
<$25,000	64.1	44.6	28.0	17.9	30.2
$25-<$50,000	22.9	27.4	37.4	38.0	35.0
$50,000 or more	10.5	25.3	32.5	42.0	32.6
Cigarette use, %					
Current	13.5	22.2	13.9	13.9	14.4
Former	28.9	49.9	35.2	58.2	43.9
Never	57.6	27.9	50.9	27.9	41.6
Alcohol use, %					
Current	18.2	40.4	51.6	62.2	50.4
Former	36.9	43.6	25.5	28.8	29.5
Never	44.9	16.0	22.9	9.0	20.1
Diabetes mellitus, %	26.6	23.9	11.1	15.7	15.8
Hypertension, %	70.1	59.9	41.0	42.3	46.6
Coronary heart disease, %	4.5	9.5	3.8	14.7	8.4
Stroke, %	2.2	4.5	1.4	2.3	2.1
Hyperlipidemia, %	35.7	36.9	41.1	40.8	40.0
Body mass index (kg/m^2), mean ± SD	31.7 ± 6.7	28.7 ± 4.9	28.2 ± 5.9	28.4 ± 4.3	28.7 ± 5.6
APOE ε4, %	39.0	42.1	27.2	27.4	29.9
Oral health conditions					
Edentulous, %	22.5	14.0	10.9	12.8	13.4
Number of teeth[1], mean ± SD	17.2 ± 7.4	18.3 ± 7.8	23.0 ± 6.4	22.8 ± 6.8	21.9 ± 7.1
Periodontal disease[1], %					
Had periodontal pockets					
BOP > 50%	14.4	31.0	8.2	13.3	12.3
BOP 10- ≤ 50%	21.9	27.4	39.6	47.4	39.5
BOP < 10%	8.7	12.4	19.9	20.5	18.5
No periodontal pockets					
BOP ≥ 10%	22.8	16.0	16.0	11.4	14.9
BOP < 10%	32.2	13.2	16.3	7.4	14.4
Cognitive functions					
Delayed word recall, mean ± SD	6.3 ± 1.6	5.6 ± 1.7	7.0 ± 1.5	6.3 ± 1.5	6.6 ± 1.6
Digit symbol substitution, mean ± SD	32.3 ± 13.2	28.9 ± 13.2	49.7 ± 11.0	43.9 ± 10.7	43.8 ± 13.3
Word fluency, mean ± SD	29.1 ± 12.7	27.4 ± 14.1	36.1 ± 11.6	33.5 ± 12.3	33.6 ± 12.5

n, total number of study group; SD, standard deviation; BGI, Biofilm-Gingival Interface
BGI classified periodontal disease based on probing pocket depth (PPD) and the extent of bleeding on probing (BOP).
[1]Only among dentate participants who received periodontal examination (n = 5,942)

Table 4-2. Comparisons of self-reported causes of tooth loss, the use of prosthesis, and gum disease of study participants who were edentulous and those who were dentate

Self-reported	Dental status	
	Edentulous (n = 1,320)	Dentate (n = 8,554)
Causes of tooth loss		
Loss due to cavities[1], %	73.6	71.8
Unknown	1.9	3.4
Missing	0.08	0.01
Loss due to gum disease[1], %	32.5	11.1
Unknown	3.3	2.0
Missing	0.08	0.01
Loss due to wisdom teeth[1], %	75.9	75.1
Unknown	2.6	3.4
Missing	0.08	0.05
Loss due to overcrowding[1], %	9.2	18.6
Unknown	2.0	2.2
Missing	0.08	0.01
Loss due to other reasons[1], %	18.6	14.8
Unknown	1.2	0.6
Missing	0.2	0.1
Prosthesis use		
Had false teeth[1], %	97.8	50.5
Missing	0.2	0.05
Age when got first false teeth[2]		
Mean ± SD	36.0 ± 13.7	38.6 ± 14.6
Gum disease		
Ever noticed any loose teeth, %	26.1	15.7
Unknown	1.8	0.2
Missing	2.2	1.0
Had gum disease, %	22.0	22.3
Unknown	0.3	0.5
Missing	0	0.06
Years of having gum disease[3]		
Mean ± SD	24.3 ± 12.9	11.1 ± 10.1
<10 years, %	10.7	51.0
10- <20 years, %	23.1	27.0
20- <30 years, %	24.1	14.8
≥ 30 years, %	42.1	7.2

n, total number of study group; SD, standard deviation

[1]Of 1,290 edentulous and 7,698 dentate participants who reported history of tooth loss.

[2]Of 1,261 edentulous and 3,886dentate participants who had prosthesis, 1,202 edentulous and 3,683 dentate participants reported age when they got first false tooth.

[3]Of 291 edentulous and 1,907 dentate participants who had gum disease, 290 edentulous and 1,985 dentate participants reported duration since they were firstly diagnosed.

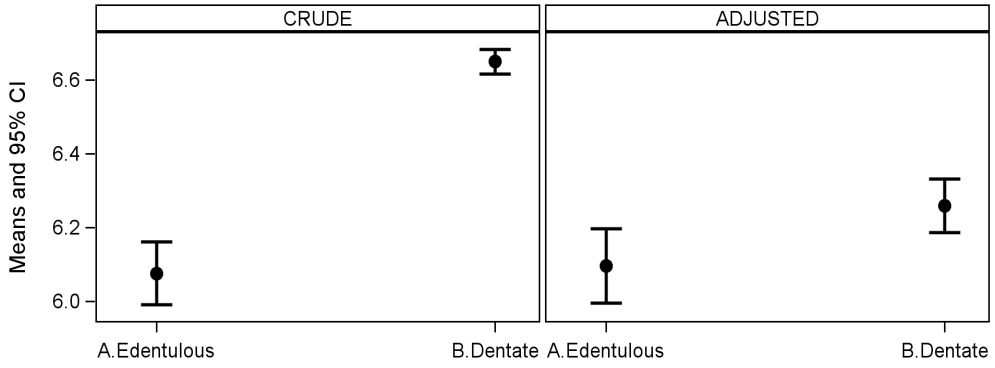
Table 4-3**.** Regression coefficients for the associations between oral health measures and Visit 4 cognitive scores

		Delayed word recall		Digit symbol substitution		Word fluency	
	n	b (SE)	*P-value*	b (SE)	*P-value*	b (SE)	*P- value*
Dental status	9,874						
Edentulous	1,320	-0.16 (0.046)	**0.0004**	-2.18 (0.30)	**<0.0001**	-1.87 (0.35)	**<0.0001**
Dentate	8,554	Ref		Ref		Ref	
Periodontal disease	5,942						
Had periodontal pockets							
BOP > 50%	733	0.047(0.076)	0.4493	-0.44 (0.48)	**0.0464**	-0.78 (0.59)	**0.0015**
BOP 10- ≤ 50%	2,374	0.069 (0.060)		0.26 (0.38)		0.12 (0.46)	
BOP < 10%	1,097	0.047 (0.069)		0.21 (0.44)		1.31 (0.53)	
No periodontal pockets							
BOP ≥ 10%	884	0.13 (0.071)		-0.84 (0.45)		-0.78 (0.55)	
BOP < 10%	854	Ref		Ref		Ref	
Number of teeth	5,942						
1-tooth increase		0.0024 (0.003)	0.4252	0.069 (0.019)	**0.0003**	0.086 (0.023)	**0.0002**

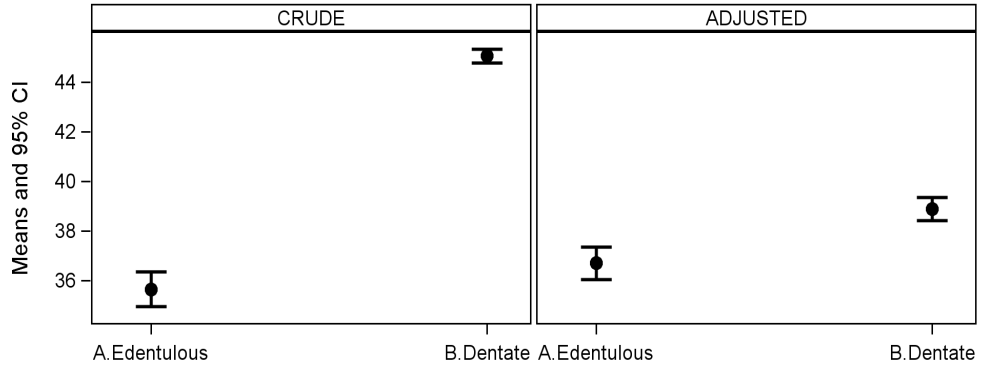
b, regression coefficient; SE, standard error; BGI; Biofilm-Gingival Interface
BGI classified periodontal disease based on probing pocket depth (PPD) and the extent of bleeding on probing (BOP).
Covariates in models included age, gender, race-center, education, income, smoking, alcohol use, and diabetes.

I. Figures

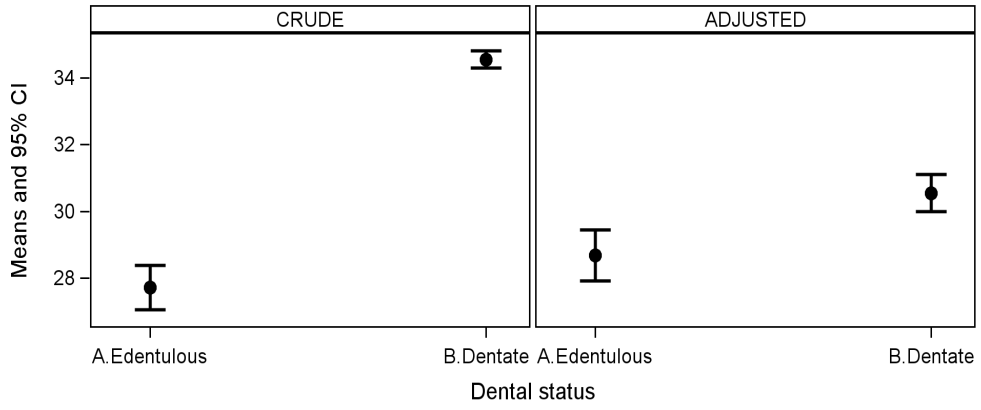
a. Delayed word recall



b. Digit symbol substitution



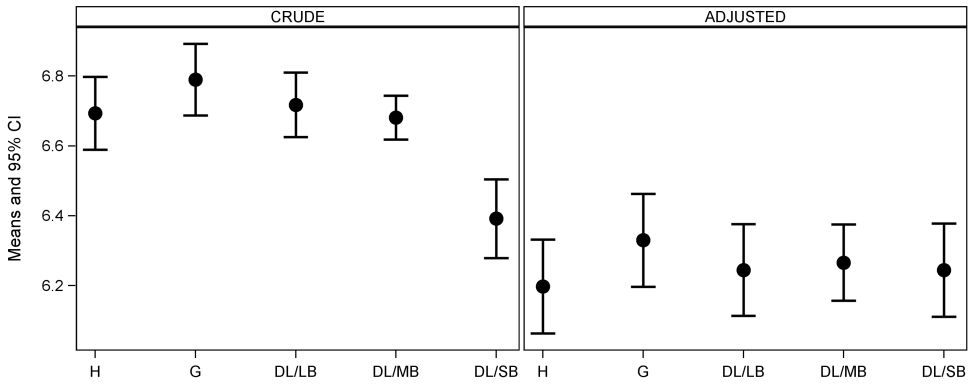
c. Word fluency



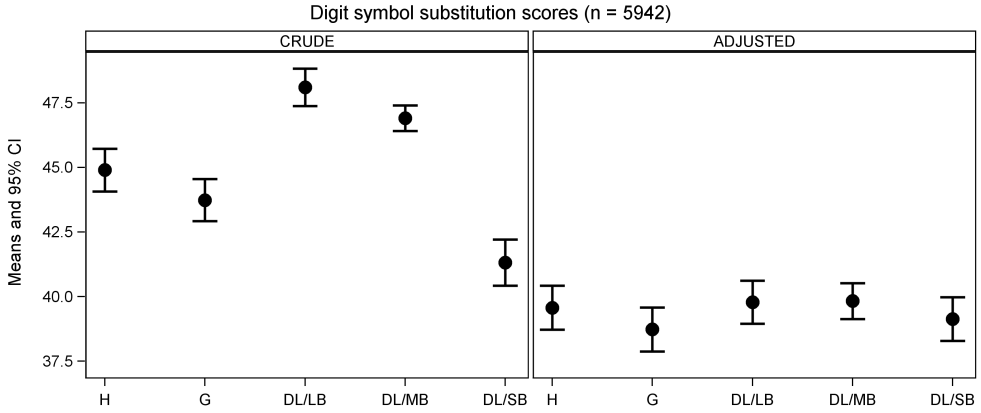
Covariates in the adjusted model included age, gender, race-center, education, income, smoking, alcohol use, and diabetes

Figure 4-1. Crude and adjusted means with 95% confidence intervals of three cognitive scores, comparing edentulous participants with dentate participants (n = 9,874)

a. Delayed word recall



b. Digit symbol substitution



c. Word fluency

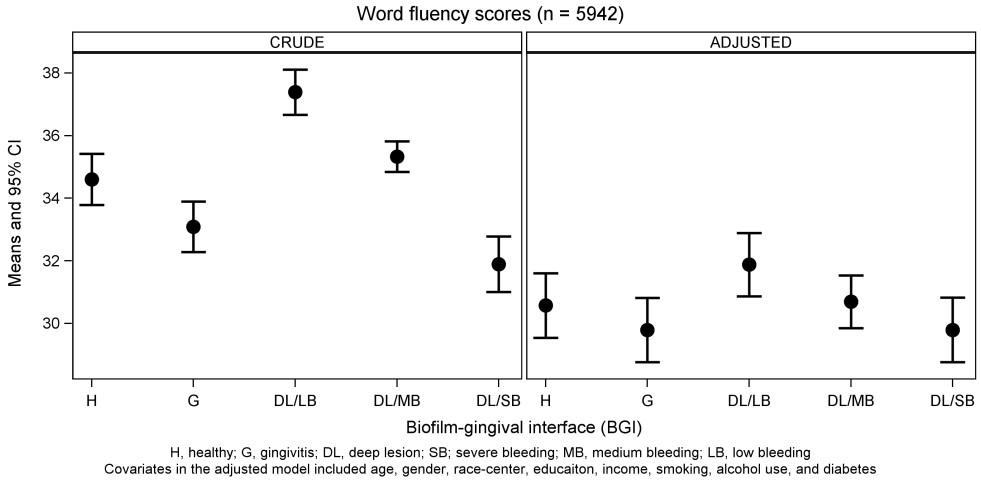


Figure 4-2. Crude and adjusted means with 95% confidence intervals of three cognitive scores, comparing among five levels of periodontal conditions (n = 5,942)

J. Supplemental materials

Covariates definition and classification

Diabetic status was determined by fasting plasma glucose ≥ 126 mg/dL, non-fasting plasma glucose ≥ 200 mg/dL, self-reported- history of physician-diagnosed diabetes or current medication for diabetes. Hypertension was defined as a previous diagnosis of hypertension, taking hypertensive medication, or having a current systolic blood pressure of 140 mmHg or higher or a diastolic blood pressure 90 mmHg. Stroke was defined as a self-reported history of physician-diagnosed stroke or stroke validated by an ARIC clinician through a review of medical records. Coronary heart disease was defined as adjudicated myocardial infraction on the electrocardiogram at baseline, or prior self-reported history of myocardial infarction, coronary artery bypass surgery or angioplasty.

Effect measure modification

We also tested whether diabetes and APOE ε4 were the effect measure modifiers by examining likelihood ratio for type 3 analysis and stratum-specific estimates. If the *p-value* is less than 0.10 or stratum-specific estimates were substantially difference, the interaction terms between oral health measures and diabetes or APOE ε4 are included in the regression model. In this study sample, no effect measure modification by diabetes and APOE ε4 was observed.

K. Supplemental Tables

Table 4-4. Self-reported causes of tooth loss, prosthesis use, gum disease, oral hygiene care, and dental visits of dentally-screened participants

Self-reported	African American		White		
	Female (n = 1,301)	Male (n = 693)	Female (n = 4,209)	Male (n = 3,671)	All (n = 9,874)
Causes tooth loss	95.4	94.8	88.8	91.3	91.0
Lost any natural teeth, %					
Missing	3.4	3.6	0.6	0.4	1.1
Loss due to cavities[1], %	86.3	85.3	66.3	70.3	72.0
Unknown	1.5	1.9	3.6	3.5	3.1
Missing	0	0.1	0.03	0	0.02
Loss due to gum disease[1], %	22.4	18.7	11.9	12.8	14.2
Unknown	2.7	5.6	1.5	2.2	2.2
Missing	0	0.1	0.03	0	0.02
Loss due to wisdom teeth[1], %	80.5	70.6	75.4	74.0	75.2
Unknown	2.4	2.6	3.0	4.1	3.3
Missing	0.1	0.3	0.05	0	0.1
Loss due to overcrowding[1], %	4.9	6.2	21.9	18.9	17.3
Unknown	1.7	2.3	2.1	2.5	2.2
Missing	0	0.1	0.03	0	0.02
Loss due to other reasons[1], %	6.9	7.3	19.4	15.4	15.3
Unknown	0.4	0.9	0.7	0.7	0.7
Missing	0.08	0.1	0.1	0.09	0.1
Prosthesis use					
Had false teeth[1], %	71.0	53.8	55.0	55.3	57.3
Missing	0	0.1	0.1	0.03	0.07
Age when got first false teeth[2]					
Range (years)	72 - 13	72 - 14	73 - 10	74 - 8	74 - 8
Mean ± SD	41.4 ± 12.7	45. 7 ± 13.9	37.0 ± 14.2	38.0 ± 15.4	38.6 ± 14.6
Ever noticed any loose teeth, %	21.5	27.4	13.7	17.5	17.1
Unknown	0.8	0.3	0.4	0.5	0.4
Missing	3.5	3.7	0.7	0.5	1.2
Gum disease					
Had gum disease, %	22.4	20.9	22.5	22.2	22.3
Unknown	0.5	0.4	0.5	0.4	0.5
Missing	0.1	0.1	0.02	0.03	0.05
Years of having gum disease[3]					
Range (years)	0 - 48	0 - 53	0 -53	0 - 55	0 - 55
<10 years, %	57.1	62.1	43.4	41.4	45.7
10- <20 years, %	22.8	22.1	26.0	29.1	26.5
20- <30 years, %	11.1	8.6	16.7	18.3	16.0
≥ 30 years, %	9.0	7.1	13.8	11.2	11.8
Oral hygiene care					
Brushing teeth[4]					
None	1.6	2.5	0.5	2.7	1.6
Once a day	22.9	40.8	17.9	37.8	27.5
Twice or more a day	72.7	53.5	81.0	59.0	69.9
Missing	2.8	3.2	0.6	0.5	1.0
Dental flossing[4]					
None	43.5	59.7	22.0	43.9	35.4
Once a week	7.7	8.6	6.6	9.7	8.0
Twice or more a week	45.9	28.4	70.8	45.9	55.6
Missing	2.8	3.3	0.6	0.5	1.0

Dental visits					
Last time saw dentist[4]					
> 36 months	24.3	24.3	4.9	7.5	9.5
12-<36 months	24.6	24.0	8.3	11.7	12.6
<12 months	47.8	48.2	86.1	80.3	76.8
Missing	3.3	3.5	0.7	0.5	1.1
Reasons to see dentist[4]					
Do not see a dentist	4.2	4.7	0.6	0.5	1.3
Only when having problems	57.5	61.7	13.4	22.5	25.4
A regular basis	35.5	30.2	85.4	76.5	72.3
Missing	2.8	3.4	0.6	0.5	1.0

n, total number of study group; SD, standard deviation
[1]Of 8,988 participants who reported tooth loss.
[2]Of 5,147 participants who had prosthesis, 4,885 reported age when they got first false tooth.
[3]Of 2,198 participants who had gum disease, 2,185 reported duration since they were firstly diagnosed.
[4]Only among dentate participants (n = 8,554)

Table 4-5. Cognitive scores at Visit 4 in relation to selected study characteristics (n = 9,874)

Characteristics	Col %	Delayed word recall		Digit symbol substitution		Word fluency	
		b (SE)	*P-value*	b (SE)	*P-value*	b (SE)	*P-value*
Age at Visit 4 (years)							
> 65	34.3	-0.73 (0.038)	<0.0001	-7.52 (0.31)	<0.0001	-2.49 (0.30)	<0.0001
60-65	31.8	-0.24 (0.039)		-2.70 (0.32)		-1.42 (0.31)	
51-59	33.9	Ref		Ref		Ref	
Gender							
Male	44.2	-0.67 (0.031)	<0.0001	-4.12 (0.27)	<0.0001	-1.90 (0.25)	<0.0001
Female	55.8	Ref		Ref		Ref	
Race							
African American	20.2	-0.64 (0.039)	<0.0001	-15.87 (0.29)	<0.0001	-6.35 (0.31)	<0.0001
White	79.8	Ref		Ref		Ref	
Study sites							
Forsyth	25.3	0.21 (0.043)	<0.0001	0.20 (0.32)	<0.0001	-0.57 (0.34)	<0.0001
Jackson	17.8	-0.54 (0.048)		-14.99 (0.36)		-5.80 (0.37)	
Minneapolis	29.1	0.17 (0.042)		3.79 (0.31)		2.51 (0.33)	
Washington	27.7	Ref		Ref		Ref	
Education							
Less than high school	17.6	-0.90 (0.045)	<0.0001	-16.70 (0.34)	<0.0001	-14.44 (0.33)	<0.0001
High school completion	42.7	-0.20 (0.034)		-4.16 (0.26)		-6.28 (0.25)	
Post-secondary education	39.7	Ref		Ref		Ref	
Income							
Refused	2.2	-0.67 (0.11)	<0.0001	-9.40 (0.85)	<0.0001	-6.41 (0.84)	<0.0001
<$25,000	30.2	-0.74 (0.04)		-13.61 (0.31)		-9.00 (0.30)	
$25-< $50,000	35.0	-0.32 (0.038)		-5.20 (0.30)		-3.57 (0.29)	
$50,000 or more	32.6	Ref		Ref		Ref	
Cigarette use							
Current	14.4	-0.24 (0.049)	<0.0001	-4.16 (0.41)	<0.0001	-1.87 (0.38)	<0.0001
Former	43.9	-0.19 (0.034)		-0.58 (0.29)		0.40 (0.27)	
Never	41.6	Ref		Ref		Ref	
Alcohol use							
Current	50.4	0.28 (0.042)	<0.0001	7.96 (0.34)	<0.0001	5.95 (0.32)	<0.0001
Former	29.5	-0.015 (0.046)		1.42 (0.37)		1.54 (0.36)	
Never	20.1	Ref		Ref		Ref	
Diabetes mellitus							
Yes	15.8	-0.45 (0.044)	<0.0001	-6.29 (0.36)	<0.0001	-3.86 (0.34)	<0.0001
No	84.2	Ref		Ref		Ref	
Hypertension							
Yes	46.6	-0.31 (0.032)	<0.0001	-5.33 (0.26)	<0.0001	-2.57 (0.25)	<0.0001
No	53.4	Ref		Ref		Ref	
Coronary heart disease							
Yes	8.4	-0.51 (0.057)	<0.0001	-4.70 (0.48)	<0.0001	-2.69 (0.45)	<0.0001
No	91.6	Ref		Ref		Ref	
Stroke							
Yes	2.1	-1.04 (0.11)	<0.0001	-10.84 (0.93)	<0.0001	-5.08 (0.87)	<0.0001
No	97.9	Ref		Ref		Ref	
Hyperlipidemia							
Yes	40.0	-0.045 (0.032)	0.1689	-0.31 (0.27)	0.2554	-0.53 (0.26)	0.0391
No	60.0	Ref		Ref		Ref	
Body mass index							
≥ 30 kg/m^2	34.4	-0.088 (0.034)	0.0092	-2.54 (0.28)	<0.0001	-1.62 (0.26)	<0.0001
< 30 kg/m^2	65.6	Ref		Ref		Ref	
APOE ε4							
Yes	29.9	-0.17 (0.035)	<0.0001	-2.27 (0.29)	<0.0001	-0.57 (0.27)	0.0385
No	70.1	Ref		Ref		Ref	

Oral health conditions							
Edentulous							
Yes	13.4	-0.58 (0.046)	<0.0001	-9.39 (0.38)	<0.0001	-6.86 (0.36)	<0.0001
No	86.6	Ref		Ref		Ref	
Number of teeth[1]							
1-24	50.4	-0.39 (0.04)	<0.0001	-6.66 (0.31)	<0.0001	-4.25 (0.31)	<0.0001
≥ 25	49.6	Ref		Ref		Ref	
Periodontal disease[1]							
Had periodontal pockets							
BOP > 50%	12.3	-0.30 (0.078)	<0.0001	-3.58 (0.62)	<0.0001	-2.71 (0.61)	<0.0001
BOP 10- ≤ 50%	39.9	-0.012 (0.062)		2.01 (0.49)		0.73 (0.49)	
BOP < 10%	18.5	0.024 (0.071)		3.20 (0.56)		2.78 (0.56)	
No periodontal pockets							
BOP ≥ 10%	14.9	0.096 (0.075)		-1.16 (0.59)		-1.51 (0.59)	
BOP < 10%	14.4	Ref		Ref		Ref	

n, total number of study group; BGI, Biofilm-Gingival Interface
BGI classified periodontal disease based on probing pocket depth (PPD) and the extent of bleeding on probing (BOP).
[1]Only among dentate participants who received periodontal examination (n = 5,942)

Table 4-6. Study centers-specific estimates for association between oral health measures and cognitive function

	Forsyth County			Jackson			Minneapolis			Washington County			All		
	b	SE	*P-value*	b	SE	*P-value*	b	SE	*P-value*	b	SE	*P-value*	b	SE	*P-value*
Delayed word recall															
		n = 2,503			n = 1,758			n = 2,878			n = 2,375				
Edentulous	-0.27	0.10	**0.0055**	-0.12	0.10	0.2366	-0.071	0.12	0.5533	-0.20	0.08	**0.0077**	-0.16	0.046	**0.0004**
BGI		n = 1,568			n = 901			n = 1,974			n = 1,499				
BGI-DL/SB	-0.057	0.14	0.4040	0.16	0.16	0.1845	-0.28	0.27	0.3399	0.053	0.18	0.9192	0.047	0.076	0.4493
BGI-DL/MB	0.012	0.12		0.34	0.15		-0.08	0.10		0.072	0.16		0.069	0.06	
BGI-DL/LB	-0.09	0.17		0.29	0.19		-0.04	0.10		-0.045	0.22		0.047	0.069	
BGI-G	0.14	0.12		0.24	0.15		0.29	0.21		-0.0031	0.19		0.132	0.076	
Number of teeth	-0.045	0.0068	0.2074	0.0001	0.01	0.9918	0.006	0.006	0.3352	0.002	0.005	0.7132	0.0024	0.003	0.4252
Digit symbol substitution															
		n = 2,503			n = 1,758			n = 2,878			n = 2,375				
Edentulous	-2.65	0.62	**<0.0001**	-1.34	0.64	**0.0383**	-1.84	0.77	**0.0167**	-2.40	0.4879	**<.0001**	-2.18	0.30	**<0.0001**
BGI		n = 1,568			n = 901			n = 1,974			n = 1,499				
BGI-DL/SB	-1.93	0.86	**0.0411**	0.51	1.04	0.7117	-1.81	1.71	**0.0255**	-0.24	1.12	0.7203	-0.44	0.48	**0.0464**
BGI-DL/MB	0.043	0.73		0.80	0.94		0.14	0.62		-0.07	1.03		0.26	0.38	
BGI-DL/LB	0.78	1.06		0.87	1.21		-0.04	0.62		-1.17	1.38		0.21	0.44	
BGI-G	-0.60	0.73		-0.43	0.96		-3.98	1.38		-0.84	1.18		-0.84	0.45	
Number of teeth	0.17	0.04	**<0.0001**	0.038	0.045	0.3933	0.046	0.037	0.2089	0.061	0.035	0.0794	0.069	0.019	**0.0003**
Word fluency															
		n = 2,503			n = 1,758			n = 2,878			n = 2,375				
Edentulous	-2.47	0.73	**0.0007**	-2.37	0.69	**0.0006**	-1.73	0.91	0.0573	-1.01	0.583	0.0828	-1.87	0.35	**<0.0001**
BGI		n = 1,568			n = 901			n = 1,974			n = 1,499				
BGI-DL/SB	-1.40	1.05	0.3248	0.30	1.15	0.4707	-5.00	2.09	**0.0061**	-0.97	1.41	0.3496	-0.78	0.59	**0.0015**
BGI-DL/MB	0.15	0.88		0.91	1.04		-0.09	0.76		-0.89	1.30		0.12	0.46	
BGI-DL/LB	0.41	1.29		2.19	1.34		1.23	0.75		0.87	1.73		1.31	0.53	
BGI-G	-0.84	0.89		-0.059	1.06		-0.34	1.68		-2.02	1.49		-0.78	0.55	
Number of teeth	0.15	0.045	**0.0007**	0.015	0.049	0.7611	0.083	0.045	0.0664	0.10	0.043	**0.0286**	0.086	0.023	**0.0002**

b , regression coefficient; SE, standard error; BGI, Biofilm-Gingival Interface

BGI classified periodontal disease based on probing pocket depth (PPD) and the extent of bleeding on probing (BOP). Five levels of BGI were the followings: BGI-DL/SB (deep lesion/severe bleeding), BGI-DL/MB (deep lesion/moderate bleeding), BGI-DL/LB (deep lesion/low bleeding), or BGI-G (gingivitis), and BGI-H (healthy).

Table 4-7. Prevalence of periodontal disease classified by CDC/AAP index and other clinical measures of periodontal disease in relation to five levels of Biofilm-Gingival Interface

	Biofilm-Gingival Interface BGI) classification[2]										All (n = 5,942)	
	No periodontal pockets				Had periodontal pockets							
	BOP = < 10%		≥ 10%		BOP = < 10%		10- ≤ 50%		> 50%			
CDC/AAP (n, col %)												
Healthy/ Mild	705	82.5	723	81.8	377	34.4	618	26.0	51	7.0	2,474	41.6
Moderate	149	6.2	161	18.2	588	53.6	1,239	52.2	297	40.5	2,434	41.0
Severe	0	0	0	0	132	12.0	517	21.8	385	52.5	1,034	17.4
Other clinical measures (mean, SD)												
Probing pocket depth	1.5	0.2	1.5	0.3	1.8	0.3	2.0	0.4	2.7	0.8		
Extent of BOP	3.3	3.2	30.2	18.9	4.6	3.0	26.1	11.0	70.8	16.1		
Attachment loss (AL)	1.4	0.7	1.4	0.7	1.6	0.8	1.7	0.8	2.9	1.5		
Extent of AL ≥ 3 mm	11.9	16.5	13.3	17.9	21.1	19.4	23.3	19.8	48.1	28.1		
Extent of plaque deposit	30.5	39.2	45.2	41.3	25.7	29.4	42.7	34.6	71.3	33.8		

CDC/AAP: The Centers for Disease Control and Prevention/ The American Academy of Periodontology; BOP, bleeding on probing

Table 4-8. Regression coefficients for associations of CDC/AAP periodontal disease classification and clinical signs of periodontal disease with three measures of cognitive function at Visit 4

		Delayed word recall		Digit symbol substitution		Word fluency	
	n (%)	b (SE)	*P-value*	b (SE)	*P-value*	b (SE)	*P-value*
CDC/AAP[1]	5,942						
Severe	2,474 (41.6)	-0.03 (0.06)	0.7704	-0.49 (0.36)	0.1717	-0.58 (0.44)	0.3791
Moderate	2,434 (41.0)	0.008 (0.04)		-0.48 (0.27)		-0.31(0.33)	
Healthy / Mild	1,034 (17.4)	Ref		Ref		Ref	
Extent of AL ≥ 3 mm	5,642	-0.002 (0.0009)	**0.0271**	-0.021 (0.006)	**0.0004**	-0.014 (0.007)	**0.0461**
Pocket depths	5,942						
≥ 4 mm	3,945 (70.0)	-0.02 (0.04)	0.6495	0.37 (0.28)	0.1869	0.54 (0.34)	0.1135
< 4 mm	1,693 (30.0)	Ref		Ref		Ref	
Gingival bleeding	5,942						
≥ 50%	859 (14.5)	0.038 (0.06)	0.4363	-0.95 (0.41)	**0.0325**	-1.51 (0.50)	**0.0073**
10- < 50%	3,132 (52.7)	0.057 (0.04)		0.019 (0.28)		-0.75 (0.34)	
0 - < 10%	1,951 (32.8)	Ref		Ref		Ref	
Plaque deposit[1]	5,638						
≥ 80%	1,346 (23.9)	-0.14 (0.06)	**0.0085**	-1.95 (0.40)	**<0.0001**	-1.93 (0.48)	**0.0002**
30-<80%	1,508 (26.7)	-0.0077 (0.05)		0.63 (0.34)		-0.12 (0.41)	
10-<30%	1,117 (19.8)	0.096 (0.06)		0.13 (0.36)		0.057 (0.43)	
0-<10%	1,667 (29.6)	Ref		Ref		Ref	

b , regression coefficient; SE, standard error; CDC/AAP: The Centers for Disease Control and Prevention/ The American Academy of Periodontology; AL, attachment loss

[1]Extent of plaque deposit was defined as a percentage of visible plaque on buccal surfaces.

Covariates in the models included age, gender, race-center, education, income, smoking, alcohol use, and diabetes.

Table 4-9. Regression coefficients for associations between dental status (complete tooth loss *vs.* dentate) and three cognitive scores at Visit 4 (n = 9,874)

	Delayed word recall			Digit symbol substitution			Word fluency		
	b	SE	*P-value*	b	SE	*P-value*	b	SE	*P-value*
Crude	-0.573	0.047	**<0.0001**	-9.394	0.382	**<0.0001**	-6.844	0.363	**<0.0001**
Model A[1]	-0.201	0.046	**<0.0001**	-2.776	0.297	**<0.0001**	-2.096	0.344	**<0.0001**
Model B[2]	-0.163	0.046	**0.0004**	-2.176	0.298	**<0.0001**	-1.868	0.347	**<0.0001**
Model C[3]	-0.162	0.046	**0.0004**	-2.166	0.298	**<0.0001**	-1.848	0.347	**<0.0001**
Change (%)[4]	-0.10			-1.06			-2.02		

b , regression coefficient; SE, standard error

Linear regression models estimated associations between complete tooth loss and cognitive scores.

[1]Adjusting for socio-demographic factors (age, gender, race-center, education, and income)

[2]Adjusting for socio-demographic factors, smoking, alcohol use, and diabetes (a minimally sufficient adjustment set)

[3]Adjusting for socio-demographic factors, smoking, alcohol use, diabetes, hypertension, hyperlipidemia, body mass index, and APOE ε4

[4]Absolute changes in estimates = $(b_{\text{model B}} - b_{\text{model C}}) \times 100$

Table 4-10. Regression coefficients for associations between periodontal disease classified by Biofilm-Gingival Interface (BGI) classification[1] and three cognitive scores at Visit 4 (n = 5,942)

	BGI	Delayed word recall			Digit symbol substitution			Word fluency		
		b	SE	*P-value*	b	SE	*P-value*	b	SE	*P-value*
Crude	DL/SB	-0.302	0.079	**<0.0001**	-3.583	0.619	**<0.0001**	-2.709	0.615	**<0.0001**
	DL/MB	-0.012	0.062		2.015	0.491		0.729	0.487	
	DL/LB	0.024	0.071		3.202	0.561		2.787	0.557	
	G	0.096	0.075		-1.162	0.590		-1.509	0.586	
Model A[2]	DL/SB	0.024	0.076	0.5107	-0.810	0.487	**0.0302**	-1.003	0.586	**0.0003**
	DL/MB	0.085	0.069		0.116	0.384		0.070	0.462	
	DL/LB	0.039	0.069		0.030	0.443		1.302	0.533	
	G	0.119	0.071		-0.926	0.454		-0.999	0.547	
Model B[3]	DL/SB	0.047	0.076	0.4493	-0.435	0.483	**0.0464**	-0.779	0.586	**0.0015**
	DL/MB	0.069	0.060		0.260	0.381		0.122	0.461	
	DL/LB	0.047	0.069		0.213	0.439		1.306	0.532	
	G	0.132	0.076		-0.841	0.451		-0.783	0.546	
Model C[4]	DL/SB	0.055	0.076	0.4199	-0.369	0.484	**0.0483**	-0.708	0.586	**0.0027**
	DL/MB	0.074	0.060		0.295	0.381		0.164	0.461	
	DL/LB	0.045	0.069		0.177	0.439		1.269	0.532	
	G	0.136	0.071		-0.827	0.451		-0.769	0.546	
Change (%)[5]	DL/SB	-0.80			-6.64			-7.11		
	DL/MB	-0.51			-3.44			-4.21		
	DL/LB	0.19			3.63			3.63		
	G	-0.36			-1.39			-1.46		

b , regression coefficient; SE, standard error

[1]Biofilm-gingival interface (BGI) classified periodontal disease based on probing pocket depth (PPD) and the extent of bleeding on probing (BOP). Linear regression models estimated the effects of BGI-DL/SB (deep lesion/severe bleeding), BGI-DL/MB (deep lesion/moderate bleeding), BGI-DL/LB (deep lesion/low bleeding), or BGI-G (gingivitis) compared to BGI-H (healthy).

[2]Adjusting for socio-demographic factors (age, gender, race-center, education, and income)

[3]Adjusting for socio-demographic factors, smoking, alcohol use, and diabetes (a minimally sufficient adjustment set)

[4]Adjusting for socio-demographic factors, smoking, alcohol use, diabetes, hypertension, hyperlipidemia, body mass index, and APOE ε4

[5]Absolute changes in estimates = $(b_{\text{model B}} - b_{\text{model C}}) \times 100$

Table 4-11. Regression coefficients for associations between number of teeth and three cognitive scores at Visit 4 (n = 5,942)

	Delayed word recall			Digit symbol substitution			Word fluency		
	b	SE	*P-value*	b	SE	*P-value*	b	SE	*P-value*
Crude	0.0275	0.003	**<0.0001**	0.531	0.022	**<0.0001**	0.332	0.022	**<0.0001**
Model A[1]	0.0038	0.003	0.1967	0.099	0.019	**<0.0001**	0.090	0.022	**<0.0001**
Model B[2]	0.0024	0.003	0.4252	0.069	0.019	**0.0003**	0.086	0.023	**0.0002**
Model C[3]	0.0021	0.003	0.4894	0.066	0.019	**0.0005**	0.082	0.023	**0.0003**
Change (%)[4]	0.03			0.27			0.38		

b , regression coefficient; SE, standard error

Linear regression models estimated the effect of 1-unit increase in the number of teeth on cognitive scores.

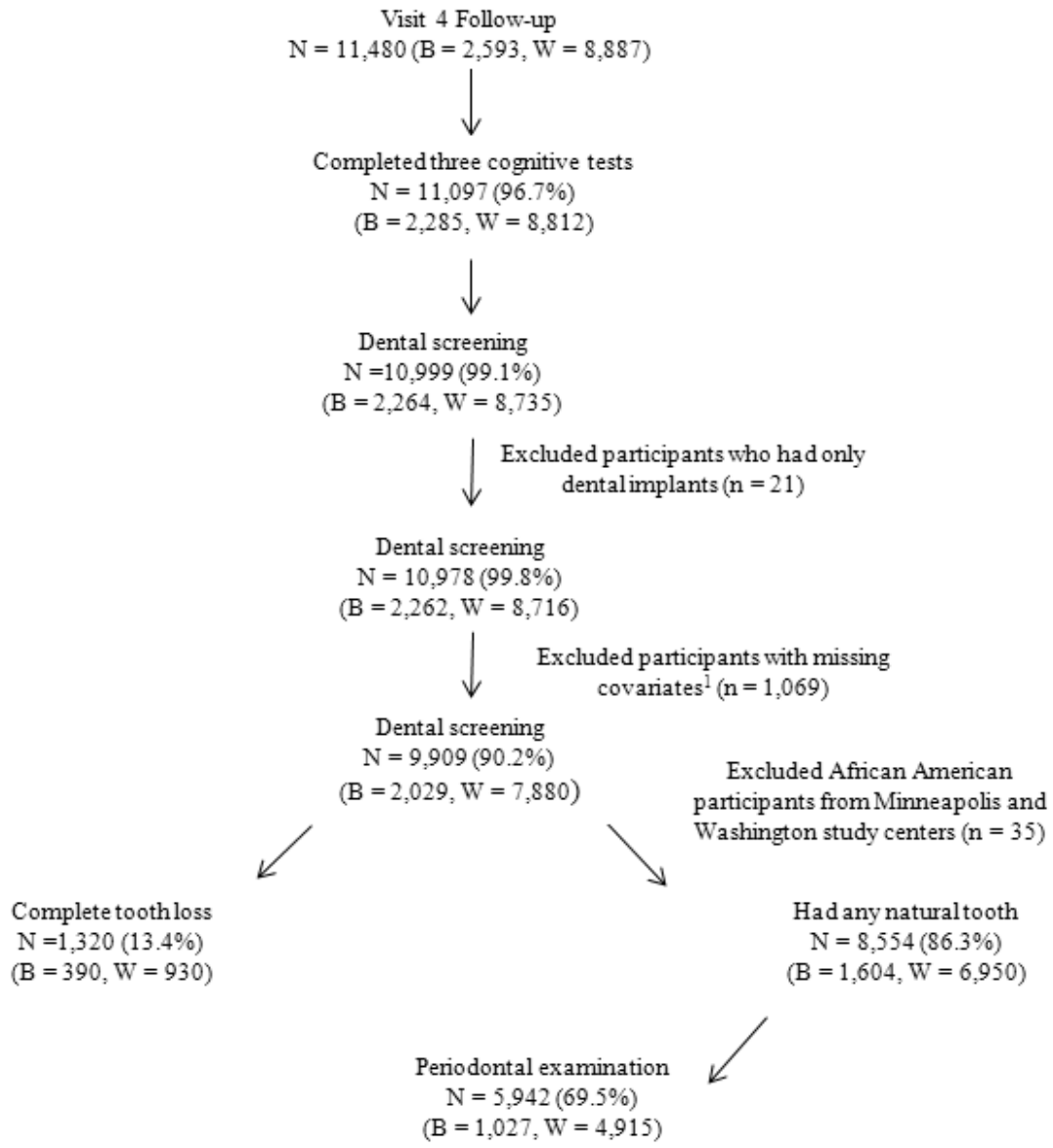
[1]Adjusting for socio-demographic factors (age, gender, race-center, education, and income)

[2]Adjusting for socio-demographic factors, smoking, alcohol use, and diabetes (a minimally sufficient adjustment set)

[3]Adjusting for socio-demographic factors, smoking, alcohol use, diabetes, hypertension, hyperlipidemia, body mass index, and APOE ε4

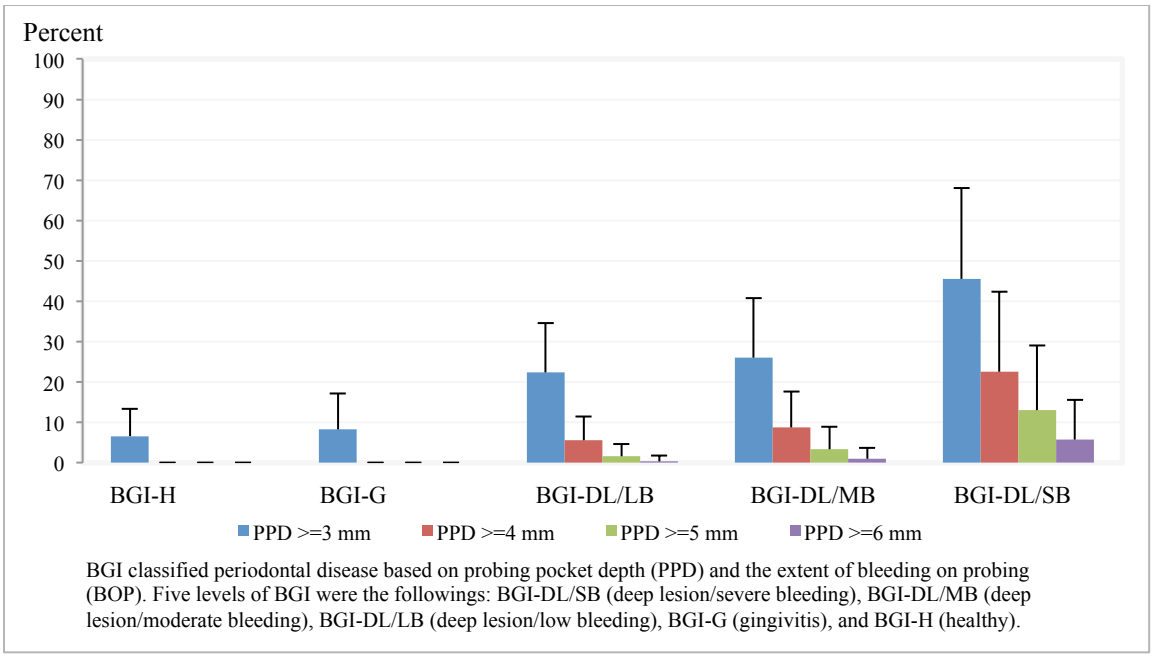
[4]Absolute changes in estimates = $(b_{\text{model B}} - b_{\text{model C}}) \times 100$

L. Supplemental figures



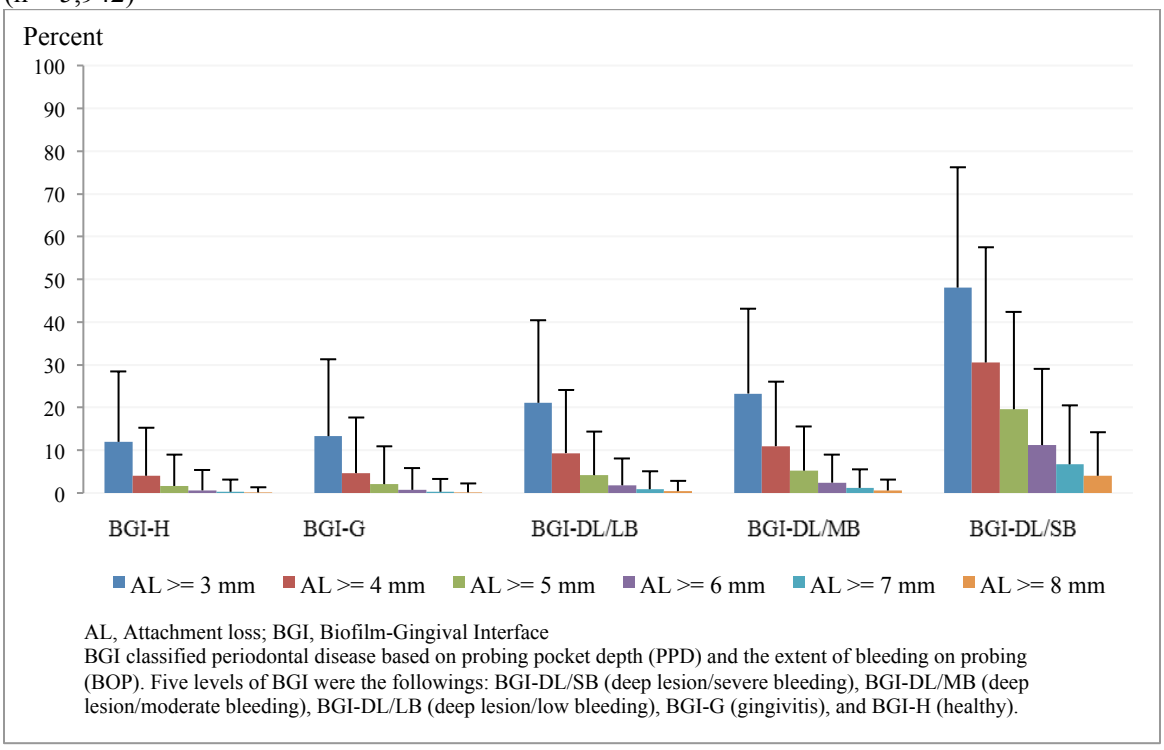
[1]Covariates: age, race, gender, study sites, education, income, smoking, alcohol use, body mass index, hyperlipidemia, diabetes, coronary heart disease, stroke, and APOE genotype.

Figure 4-3. Flow chart of ARIC participants who completed three cognitive function tests, dental screening, and comprehensive dental examination between 1996 and 1998



BGI classified periodontal disease based on probing pocket depth (PPD) and the extent of bleeding on probing (BOP). Five levels of BGI were the followings: BGI-DL/SB (deep lesion/severe bleeding), BGI-DL/MB (deep lesion/moderate bleeding), BGI-DL/LB (deep lesion/low bleeding), BGI-G (gingivitis), and BGI-H (healthy).

Figure 4-4**.** Extent of probing pocket depth by five levels of Biofilm-Gingival Interface classification (n = 5,942)



AL, Attachment loss; BGI, Biofilm-Gingival Interface
BGI classified periodontal disease based on probing pocket depth (PPD) and the extent of bleeding on probing (BOP). Five levels of BGI were the followings: BGI-DL/SB (deep lesion/severe bleeding), BGI-DL/MB (deep lesion/moderate bleeding), BGI-DL/LB (deep lesion/low bleeding), BGI-G (gingivitis), and BGI-H (healthy).

Figure 4-5**.** Extent of attachment loss by five levels of Biofilm-Gingival Interface classification (n = 5,942)

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STUDY 2: RESULTS

Tooth loss, periodontal disease, and cognitive decline in the ARIC study

A. Overview

It is inconclusive whether poor oral health in midlife increases risk for subsequent cognitive decline in later life because the majority of evidence comes from cross-sectional studies of older adults, whose cognitive function has already declined. The purpose of the present prospective study was to investigate whether poor oral health in midlife predicted eight-year cognitive function change in adults aged 52-75 years at Visit 4 (1996-1998) in the Atherosclerosis Risk in Communities (ARIC) study. Participants included a subset of ARIC participants from two study sites: Forsyth County NC and Jackson MS. All subjects completed cognitive function assessments from Visit 4 and 2004-2006 as part of the Brain MRI study, and the same subjects received a dental examination at Visit 4. Cognitive assessment consisted of Delayed Word Recall (DWR), Digit Symbol Substitution (DSS), and Word Fluency (WF) tests. At Visit 4, 4,737 participants answered screening questions, and 2,728 of 4,004 dentate participants received comprehensive oral examinations, including periodontal probing. Measures of oral health included dental status, number of teeth, and periodontal disease classified by the Biofilm-Gingival Interface (BGI) classification. From 2004-2006, cognitive function for 911 dentally screened participants was reevaluated. The generalized estimating equations (GEE) method was used to analyze repeated measures of cognitive scores with adjustment for socio-demographic characteristics and cardiovascular risk factors. Of 911 study participants, 126 (13.8%) were edentulous and 558 of 785 dentate participants received periodontal examination. About 13 % of dentally examined participants had deep periodontal lesions with severe bleeding.

At Visit 4 DWR and WF scores were lower in edentulous compared to dentate people, whereas other oral health measures were not associated with cognitive function. Mean values declined over time for all three cognitive measures, although oral health measures were not associated with degree of decline in cognitive function. In these late-middle aged adults, complete tooth loss was significantly associated with lower cognitive performance. However, neither edentulism, number of teeth, nor periodontal disease predicted greater subsequent cognitive decline.

B. Introduction

Cognitive function changes throughout one's life. These changes can be physiological or pathological in one or more cognitive domains (1,2). Emerging evidence has linked tooth loss and periodontal disease to a greater age-related cognitive decline and to neurodegenerative diseases, such as Alzheimer's disease (AD) (3-9). An inflammatory model, based on the fundamental theory that chronic periodontal disease is a complex interaction between bacterial pathogens and the host inflammatory response, has been proposed to explain the observed associations (10).

However, there is considerable uncertainty concerning the mechanisms that could lead to associations between poor oral health and cognitive function, since most studies have been cross-sectional and conducted primarily in older adults (4,8,9,11-17). For example, poor nutritional status resulting from tooth loss may affect cognitive ability (17,18). Cognitive dysfunction may lead to poorer self-care, thereby worsening oral health status (19). Low cognitive ability in early life may lead to socioeconomic inequalities in oral health (20).

Whereas many studies report associations of fewer teeth and complete tooth loss with poorer cognitive function (3,5,13,21-23), evidence regarding the association between periodontal disease and cognitive ability is mixed (3,6-8,13,14). Furthermore, periodontal disease exposure in earlier studies was defined based on clinical signs that do not provide much information about the underlying biology of periodontal disease. For instance, Stein et al. (2007) found that a low number of teeth (0-

9), but not alveolar bone loss, was associated with the incidence of dementia during 12 years of follow-up (5). In contrast, another prospective study in dentate men found that a higher rate of tooth loss, along with the progression of alveolar bone loss or probing pocket depth, increased the risk of low cognitive scores. In that study, however, baseline cognitive status was unavailable (3). Recently, a 15-year follow-up study in French older adults found a lower risk of dementia associated with fewer teeth among lower educated participants but noted that their surprising finding might have arisen if lack of teeth protected them from a source of chronic inflammation. In that study, the risk of dementia was not associated with masticatory function or community periodontal index (CPI) (7).

The purpose of the present study was to determine whether tooth loss and current inflammatory state of periodontal disease, as classified by the Biofilm-Gingival Interface (BGI) classification, predicted eight-year changes in cognitive function among community-dwelling, late middle-aged adults in the Atherosclerosis Risk in Communities (ARIC) study. We hypothesized that multiple tooth loss and severity of periodontal disease would predict low cognitive performance and subsequent cognitive decline.

C. Methods

Data and participants

Participants were members of the ARIC study, a prospective investigation of the etiology and natural history of atherosclerosis and clinical cardiovascular disease in four U.S. communities (Forsyth County, NC; Washington County, MD; suburban Minneapolis MN; and Jackson, MS). The Jackson cohort was comprised exclusively of African Americans. In this study, we used data from ARIC Visit 4 (1996-1998) and two ARIC ancillary studies: a) the Dental ARIC study; and b) the Brain Magnetic Resonance Imaging (MRI) study. Details on sampling and data collection procedures used in ARIC, including its ancillary studies, have been described elsewhere (24-26).

Cognitive function of ARIC participants was initially assessed between 1990 and 1992 (Visit 2), and has been repeatedly evaluated approximately every three years until Visit 4. Dental ARIC was a cross-sectional study conducted at Visit 4. From 2004-2006, a subset of participants from two study sites (Forsyth County NC and Jackson MS) received another cognitive function assessment as part of the Brain MRI study. For the present study, analyses were restricted to subjects who participated in the dental study at Visit 4 and the Brain MRI study with two cognitive assessments separated by eight years (between 1996-1998 and 2004-2006).

At Visit 4, 4,737 participants from the Forsyth County and Jackson study sites received cognitive functional assessments and a dental screening questionnaire, and 15.5% (n = 733) of these subjects reported complete tooth loss. Of 4,004 dentate participants, 2,728 (68.1%) underwent a comprehensive dental examination. From 2004-2006, the cognitive function of 911 participants was reevaluated (Figure 5-3).

Oral health measures

The cross-sectional Dental ARIC study consisted of a comprehensive dental examination, which included periodontal probing; collection of gingival crevicular fluid (GCF), dental plaque, and serum; and an interview. Persons requiring antibiotic prophylaxis for periodontal probing were excluded from the Dental ARIC study. Periodontal probing depth (PPD) and bleeding on probing (BOP) were assessed at six sites on all teeth by trained examiners.

The BGI index, based on measures of PPD (PPD ≤ 3 mm or PPD ≥ 4 mm) and extent of BOP (low, <10%; moderate, 10-<50%; and severe , ≥ 50%), were used to classify periodontal status into five levels. Subjects with PPD ≤ 3 mm were defined as periodontal healthy if BOP was less than 10% or gingivitis if BOP was 10% or more. Subjects with one or more periodontal pockets or PPD ≥ 4 mm were divided into low, moderate, or severe bleeding. BGI index, in contrast to traditional definitions of periodontal status, creates subgroups that share some common clinical signs, but they differ in

microbial components and inflammatory biomarker levels (27). This unique characteristic of the BGI enables us to study whether infection and inflammatory components of periodontal disease are related to cognitive function. The number of teeth present was also recorded during the dental examination. An individual's dental status was obtained from answers to the following items on a self-administered questionnaire: "Do you have any natural teeth?" and "Do you have any dental implants"? Participants who had only dental implants (n = 21) were excluded from the study.

Cognitive function

Outcomes of interest were scores from the following cognitive tests: a) Delayed Word Recall (DWR); b) Digit Symbol Substitution (DSS); and c) Word Fluency (WF). The DWR tests verbal learning and recent memory (28). The DSS, a test of concentration and psychomotor speed (29), and the WF, a test of expressive language, assess executive function (30). Higher scores in each of the three tests indicate better cognitive ability. All cognitive tests were administered by trained examiners. Cognitive test protocols for ARIC have been reported elsewhere (24).

Covariates

Covariates included socio-demographic factors (age, race, gender, educational level, income, and study sites), cardiovascular risk factors, apolipoprotein E (APOE) genotype, stroke, and coronary heart disease (CHD). Educational levels were classified as less than high school (<12 years), high school completion (12-16 years), or post-secondary education (17-21 years). Household income was coded as <$25,000, $25,000-$50,000, > $50,000, or refused (1996-1998 dollars). A variable representing race and ARIC filed centers was created to control for the racial, regional, and examiner differences in the ARIC cohort as the following: Forsyth/White, Forsyth/Black, and Jackson/Black. Cardiovascular risk factors included smoking and alcohol use (each recorded as never, former, or current), diabetes, hypertension, hyperlipidemia, and body mass index (BMI). APOE genotype was dichotomized as presence or absence APOE ε4 allele (Supplemental Methods).

Statistical analyses

Race- and gender-specific descriptive statistic and bivariate analyses were conducted. We used generalized estimating equations (GEE) to analyze eight-year changes in the three cognitive scores. The dependent variables were repeated measures of the DWR, DSS, and WF scores. Since time intervals between baseline and follow-up were slightly different among study participants (mean ± s.d. = 7.6 ± 1.0 years; median = 8 years), we used an indicator variable (time (*t*)) to identify whether the scores represented a baseline (*t* = 0) or follow-up (*t* = 1) measurement rather than using actual time intervals. The unstructured working correlation matrix was used to correct within-subject correlations in the analysis.

The hypothesis that oral health predicted cognitive decline involved testing the interaction between time and oral health measures, i.e., a model of $E(Y_{it} \mid \text{Oral health predictor}_{it}) = b_0 \pm b_1 * \text{Oral health predictor} \pm b_2 * t \pm b_3 * (\text{Oral health predictor} * t)$, where the hypothesis H_0: $b_3 = 0$ was tested. If *p-value* was greater than 0.10 for b_3, we concluded that oral health measures did not significantly predict cognitive decline over time.

Potential confounders were identified based on previous literature and bivariate analyses assessing the association between exposures and outcomes. We used directed acyclic graphs (DAGs) and the change-in-estimate procedure to select the adjustment variables in this study. The minimally sufficient set for adjustment included socio-demographic factors, smoking, alcohol use, and diabetes (i.e., the reduced model). Fully adjusted models consisted of variables from the reduced model, BMI, hyperlipidemia, hypertension, and APOE ε4. All covariates were included in the GEE models as time-independent factors. If regression coefficients of the reduced models did not differ from those for the fully adjusted models by greater than 10% or ± 0.1, the regression coefficients from the reduced models were presented in table results. Supplementary analyses addressed questions concerning cross-sectional associations between oral health measures and baseline cognitive scores in this study samples, study center-specific associations of oral health indicators and the 8-year change

in cognitive scores, as well as, impact of including participants with history of stroke in study sample. All statistical analyses were performed using SAS 9.3 (Cary, NC).

D. Result

Characteristics of study participants

The final sample contained 911 individuals with an average age of 64.7 ± 4.3 at baseline. Forty-nine percent of participants were African American and 61% were female. About half of study participants have never smoked and one-third have never used alcohol. There were notable differences between racial and gender groups in socio-demographic characteristics and in the prevalence of hypertension, diabetes, CHD, and stroke. About one-third of African American participants who primarily from Jackson study site compared to about 10% of whites, had less than 12 years of education. African Americans also had lower income and a higher prevalence of diabetes and hypertension. CHD and stroke were more prevalent among African American males compared to other three race-gender groups. Overall, African American subjects had poor oral health as indicated by fewer teeth, higher prevalence of complete tooth loss, or severe periodontal disease. However, gingivitis was more common among white subjects (Table 5-1).

Cognitive function

Older age, male sex, low education, low income, diabetes, hypertension, current smoking, CHD, stroke, APOE ε4, and poor oral health (complete tooth loss, few teeth, and periodontitis with severe bleeding) were associated with a low cognitive profile. The differences in cognitive scores were generally greater for race, educational attainment, and income. There was, as expected, a strong association between dental status and overall cognitive performance. On average, individuals with complete tooth loss had cognitive scores 0.62, 9.08, and 8.30 points lower than dentate participants for the DWR, DSS, and WF tests, respectively. The associations of other covariates with repeated measures of cognitive scores are summarized in the Supplemental Table 5-9.

Over a median interval of eight years between the two examinations, most participants experienced a relatively small cognitive decline with substantial between-subjects variability. Mean change in DWR, DSS, and WF scores were -0.7, -3.6, and -1.8 points, with 53.5%, 67.8%, and 58.2% declining and 23.7%, 6.5%, and 5.2% unchanged. In general, white females tended to have a greater decline in DSS scores while African Americans (males, and females) exhibited a greater decrease in WF scores. The decline in DWR scores was similar across race- and gender-specific groups (Table 5-2).

Oral health measures and cognitive function

Table 5-3 shows the regression coefficients (b) for oral health measures, time, and their interactions; parameter estimates for the first-order effects of each oral health measure can be interpreted as the magnitude of association between the Visit 4 oral health measure and cognitive decline, while interaction terms indicate the difference between oral health status groups in degree of change in cognitive function over eight years. At Visit 4, lower levels of all three cognitive function test scores were seen in subjects with complete tooth loss when compared to dentate subjects, although the differences did not reach statistical significance for the DSS test in the fully adjusted model. In contrast, periodontal disease and the number of teeth were not significantly associated with cognitive performance, either as first-order effects or as interactions with time.

Generally, cognitive decline among dentate participants tended to be slightly greater than that of edentulous subjects. However, oral health measures did not significantly modify the time-related degree of cognitive decline (Figures 5-2 – 5-6), except for the association between edentulism and DWR scores, which was marginally significant (*p-value* 0.0855 - Table 5.3). Individuals with complete tooth loss had adjusted DWR scores that declined from 6.1 (95% CI 5.8, 6.4) at baseline to 5.7 (95% CI, 5.4, 6.0) at the follow-up visit compared with 6.6 (95% CI, 6.3, 6.7) at baseline and 5.8 (95% CI, 5.6, 6.0) at the follow-up visit for dentate subjects (Figure 5-1).

Supplementary analyses showed dental status was associated with lower DWR and DSS at baseline (Table 5-4). Neither fewer teeth nor periodontal disease was associated with baseline cognitive scores of three tests. Associations between edentulism and change in cognitive scores in Forsyth study center was slightly stronger than that of Jackson study center (Table 5-6). The results were generally unchanged after excluding participants with history of stroke at baseline (Table 5-7).

E. Discussion

Four possible mechanisms have been proposed for the relation between poor oral health and lower cognitive function: (a) residual confounding by socio-demographic factors or other environmental factors; (b) nutritional deficiency resulting from tooth loss; (c) increased systemic inflammatory response (tooth loss is often a consequence of severe periodontal disease) (10,15,16); and (d) an adverse impact of cognitive decline on oral hygiene.

Our study found complete tooth loss, though not periodontal disease and number of teeth, to be associated with low performance on two cognitive tests (DWR and WF) at baseline (Visit 4). However, although all three cognitive scores declined over time, we did not find that complete tooth loss, periodontal disease, and few teeth at baseline predicted a greater cognitive decline. We also observed a smaller decline in memory function among edentulous participants compared to dentate subjects.

The most important strengths of the present study are the large population-based cohort of community-dwelling, predominantly late middle-aged adults and the quality of examination data. Cognitive assessment and periodontal examination, using a full-mouth protocol, were carried out by trained examiners. Several limitations are also relevant. Our study followed participants in only two of the four ARIC sites (Forsyth County and Jackson), and these differed greatly in regard to racial composition, socioeconomic characteristics, edentulism, and baseline cognitive scores (especially DSS and WF test scores).

Another limitation of our study is that oral health indicators were measured only at Visit 4, so that information was unavailable on the trajectory of oral health (i.e., additional tooth loss or periodontal disease progression). Thus, we cannot assess whether low cognitive performance influences oral health. Furthermore, assessments at two time points provide limited ability to differentiate true change from changes due to learning effects, random fluctuations, and measurement errors. Lastly, our study examined only three cognitive function tests, evaluating two cognitive domains. Thus, our failure to observe more rapid cognitive decline in participants with worse oral health provides limited evidence against the existence of a relationship.

Complete tooth loss:

Our findings of lower scores on Visit 4 DWR and WF for edentulous participants are consistent with previous studies which found that people with few teeth or complete tooth loss have lower cognitive function in the memory or executive function domains (3,6,13). We also observed a marginally-significantly lower score on the DSS for edentulous participants in the Forsyth County site but no association among Jackson site participants, whose cognitive function, education, and oral health measures were all markedly lower (Supplemental Tables 5-5, 5-6).

Our findings are not consistent, however, with previous studies that found edentulism to predict more rapid cognitive decline and greater incidence of dementia (6,23). In fact, our study found a *slower* cognitive decline in edentulous participants. The many differences between the communities studied in the previous studies and in ours could conceivably account for the different findings. However, when we carried out separate analyses in the Forsyth and Jackson sites, which differ substantially in numerous characteristics, we observed a smaller decline in memory function in edentulous participants in both sites; it was, if anything, stronger in Forsyth (Supplemental Table 5-6). The previous studies followed older participants, who experienced substantial cognitive declines, whereas our study population was predominantly late middle-aged adults, who had only modest

cognitive declines during the follow-up period. The smaller overall decline may have limited the opportunity to see a difference by oral health status.

Other possible explanations for why our study did not observe greater cognitive decline for participants with poorer oral health – and for our observation of a smaller decrease in DWR scores for edentulous participants than for dentate participants – relate to the narrow time window under observation. Cognitive function of edentulous participants, who were older and had poorer socio-demographic characteristics than dentate participants, may have begun to decline earlier in life, before our study baseline, and had now reached a stable level. Other possible explanations relate to the heterogeneity of age-related cognitive decline, where some cognitive domains decline earlier (i.e., memory function) and more rapidly than others, and inter-individual variability increases during the aging process (31). A longitudinal study in French elderly has reported a lower risk of dementia in lower educated participants with extensive tooth loss. The authors suggested that the observed association was possibly a result of greater tooth extraction in people with lower educational attainment, leading to better periodontal health (7). Such suppression of a source of chronic inflammation could conceivably explain why we observed a smaller decrement in cognitive function among edentulous persons.

Yet another possibility is that the cross-sectional association between poorer oral health and worse cognitive function reflects an adverse effect of low cognitive function on oral health status. One longitudinal study has suggested that the observed association between complete tooth loss and cognitive impairment in older adults results from lower cognitive ability in early life predisposing individuals to edentulism (21).

Periodontal disease:

Previous findings regarding periodontal disease and cognitive function are mixed. Some studies, including our previous cross-sectional study (Study #1), have found a significant association

between periodontal disease and low cognitive ability (3,8,14-16), whereas others have not (5-7,13). Interpretation of these findings is complicated by differences in the definition and measurement of periodontal disease. A wide range of periodontal disease measures have been used, including biological markers (14,32), attachment loss (16), periodontal pocket depth (3,13), and self-reported periodontal status (6). Also, many study populations are highly selected, and results from them may not be indicative of what would be seen in other populations (3,5,7,8,13).

In our study, BGI index was selected based on the concept that if periodontal infection and inflammation truly affect cognitive decline, we should observe a dose-response trend in the association because BGI reflects the underlying biology of periodontal disease (27). However, as noted in Chapter 4, the expected dose-response pattern might be masked by the nature of the clinical periodontal measures and effects of past treatments, none of which can be discerned given that periodontal measurements were recorded only a one visit. Furthermore, although different bacterial species are linked to gingivitis and severe periodontitis, both conditions have been correlated with elevated levels of gingival bleeding, inflammatory markers, and plaque scores.

We found some indication that participants with a greater extent of bleeding (i.e., gingivitis and severe periodontitis) at baseline (Visit 4) had lower cognitive function than those who were healthy or had a lesser extent of bleeding, but we did not observe differences in the rate of cognitive decline to be related to the value of the BGI index. Our study may have been underpowered to detect an association between BGI and cognitive decline because cognitive change during the eight-year follow-up was modest, and the mean differences between the two extreme BGI groups were relatively small. However, it is also possible that subtle changes in cognitive function occurring prior to study baseline resulted in poor oral hygiene care, thereby increasing plaque level and the extent of bleeding on probing at baseline.

Further relevance and interpretation of supplementary analyses were discussed in Chapter 6.

F. Conclusion

We have not observed a relationship between edentulism, number of teeth, or periodontal disease in middle-aged adults and cognitive decline over the subsequent 8 years. Our study may have had insufficient statistical power given the low rate of observed cognitive decline, or the relationship may manifest only at older ages and/or in relation to more severe levels of impaired cognition.

G. Human participants protection

No protocol approval was necessary because this study involved the analysis of secondary data.

H. Tables

Table 5-1. Race- and gender-specific socio-demographics, health conditions, and cognitive function at baseline (1996-1998) of study participants from Forsyth County, NC and Jackson, MS study sites

Characteristics	African American		White		All
	Female (n = 291)	Male (n = 151)	Female (n = 268)	Male (n = 201)	(n = 911)
Age at Visit 4, mean ± SD	64.0 ± 4.3	63.8 ± 4.5	65.4 ± 4.3	65.5 ± 4.1	64.7 ± 4.3
Study sites, %					
Forsyth	10.0	13.2	100	100	56.9
Jackson	90.0	86.8	0	0	43.1
Education, %					
Less than high school	32.0	31.8	8.6	9.0	20.0
High school completion	27.5	19.2	51.5	34.8	34.8
Post-secondary education	40.5	49.0	39.9	56.2	45.2
Income, %					
Refused	3.1	0.7	3.0	2.0	2.4
<$25,000	62.9	45.7	26.5	13.9	38.5
$25-<$50,000	23.7	29.1	38.0	35.3	31.4
$50,000 or more	10.3	24.5	32.5	48.8	27.7
Cigarette use, %					
Current	12.0	15.9	16.0	8.5	13.1
Former	27.8	47.0	26.5	65.2	38.8
Never	60.2	37.1	57.5	26.3	48.1
Alcohol use, %					
Current	18.9	34.4	40.3	55.2	35.8
Former	35.1	48.3	25.4	33.3	34.0
Never	46.0	17.2	34.3	11.4	30.2
Diabetes mellitus, %	25.1	21.9	7.1	13.4	16.7
Hypertension, %	67.0	51.7	32.8	39.8	48.4
Coronary heart disease %	3.1	3.3	1.9	11.1	4.6
Missing	1.4	2.0	2.2	1.5	1.8
Stroke, %	2.7	0.7	0.7	3.0	1.9
Missing	0	0.7	0	0	0.1
Hyperlipidemia, %	35.0	31.8	35.1	36.3	34.8
Body mass index (kg/m^2), mean ± SD	30.6 ± 5.4	28.2 ± 4.5	26.1 ± 4.7	27.0 ± 3.6	28.1 ± 5.0
APOE ε4, %	37.5	37.1	25.0	23.4	30.6
Oral health conditions					
Edentulous, %	23.4	15.2	7.1	8.0	13.8
Number of teeth[1], mean ± SD	16.3 ± 7.3	18.9 ± 7.9	23.7 ± 5.5	22.1 ± 7.3	20.6 ± 7.5
Periodontal disease[1], %					
Had periodontal pockets					
BOP > 50%	12.2	27.8	3.7	19.0	13.3
BOP 10- ≤ 50%	27.9	38.0	29.5	35.9	31.9
BOP < 10%	6.8	8.9	8.4	6.3	7.5
No periodontal pockets					
BOP ≥ 10%	24.5	13.9	39.5	25.4	28.3
BOP < 10%	28.6	11.4	18.9	13.4	19.0

n, total number of study group; SD, standard deviation; BGI, Biofilm-gingival index
BGI classified periodontal disease based on probing pocket depth (PPD) and the extent of bleeding on probing (BOP).
[1]Only among dentate participants who received periodontal examination (n = 558)

Table 5-2. Cognitive scores at Visit 4 and the eight-year changes in cognitive scores

Cognitive scores, mean ± SD	African American		White		All (n = 911)
	Female (n = 291)	Male (n = 151)	Female (n = 268)	Male (n = 201)	
Cognitive scores at Visit 4					
Delayed word recall	6.5 ± 1.5	5.8 ± 1.6	7.2 ± 1.3	6.8 ± 1.5	6.7 ± 1.6
Digit symbol substitution	34.3 ± 12.5	30.0 ± 12.7	50.1 ± 9.9	44.6 ± 40.5	40.5 ± 14.0
Word fluency	30.8 ±12.6	28.9 ± 14.4	37.0 ± 11.4	35.1 ± 12.1	33.3 ± 12.9
Change in cognitive scores[1]					
Delayed word recall	-0.7 ± 1.8	-0.6 ± 1.7	-0.6 ± 1.7	-0.8 ± 1.6	-0.7 ± 1.7
Digit symbol substitution	-3.1 ± 9.1	-3.4 ± 6.9	- 4.9 ± 6.7	-2.9 ± 6.8	-3.6 ± 7.6
Word fluency	-2.5 ± 7.1	-2.5 ± 9.4	-1.6 ± 7.4	-0.7 ± 6.9	-1.8 ± 7.6

[1]Changes in cognitive scores = Scores at follow-up – Scores at baseline

Table 5-3**.** Regression coefficients for the effects of time, oral health measures, and their interaction on three cognitive scores

	n	Delayed word recall		Digit symbol substitution		Word fluency	
		b (SE)	*P-value*	b (SE)	*P-value*	b (SE)	*P-value*
Dental status[1,2]	911						
Time (F/U *vs.* Baseline)		-0.74 (0.06)	**<0.0001**	-3.76 (0.26)	**<0.0001**	-1.91 (0.28)	**<0.0001**
Edentulous		-0.42 (0.16)	**0.0357**	-1.47 (1.08)	0.2438	-3.03 (0.02)	**0.0014**
Edentulous x Time		0.29 (0.17)	0.0855	0.89 (0.93)	0.3389	0.46 (0.60)	0.4512
Periodontal disease[1]	558						
Time (F/U *vs*. Baseline)		-0.98 (0.18)	**<0.001**	-3.38 (0.84)	**<0.0001**	-1.32 (0.80)	**<0.0001**
BGI-DL/SB		-0.16 (0.21)	0.6040	-0.28 (1.56)	0.5165	-0.50 (1.79)	0.6225
BGI-DL/MB		0.0015 (0.16)		0.88 (1.18)		1.50 (1.44)	
BGI-DL/LB		0.15 (0.24)		2.38 (1.56)		1.75 (2.15)	
BGI-G		0.12 (0.16)		0.91 (1.22)		-0.75 (1.40)	
BGI-DL/SB x Time		0.45 (0.27)	0.5655	0.55 (1.14)	0.7636	-0.96 (1.17)	0.2173
BGI-DL/MB x Time		0.23 (0.22)		-0.33 (0.96)		-1.36 (1.00)	
GI-DL/LB x Time		0.10 (0.29)		0.12 (1.32)		-2.18 (1.45)	
BGI-G x Time		0.22 (0.22)		-0.64 (0.99)		0.17 (0.98)	
Number of teeth[1]	558						
Time (F/U *vs.* Baseline)		-0.67 (0.23)	**0.0048**	-3.38 (0.86)	**0.0001**	-2.72 (0.90)	**0.0028**
1-tooth increase		0.0031 (0.0086)	0.7129	0.046 (0.056)	0.3959	-0.0071 (0.073)	0.9233
1-tooth increase x Time		-0.0053 (0.01)	0.6109	-0.0094 (0.039)	0.8071	0.035 (0.0042)	0.4100

b , regression coefficient; SE, standard error; BGI, Biofilm -Gingival Interface
BGI classified periodontal disease based on probing pocket depth (PPD) and the extent of bleeding on probing (BOP).
BGI-DL/SB (deep lesion/severe bleeding), BGI-DL/MB (deep lesion/moderate bleeding), BGI-DL/LB (deep lesion/low bleeding), BGI-G (gingivitis), and BGI-H (healthy). In regression models, BGI-H was the reference group.
[1]Adjusting for age, gender, race-center, education, income, smoking, alcohol use, and diabetes
[2]Digit symbol substitution: Adjusting for age, gender, race-center, education, income, smoking, alcohol use, diabetes, hypertension, hyperlipidemia, body mass index, and APOE ε4

I. Figures

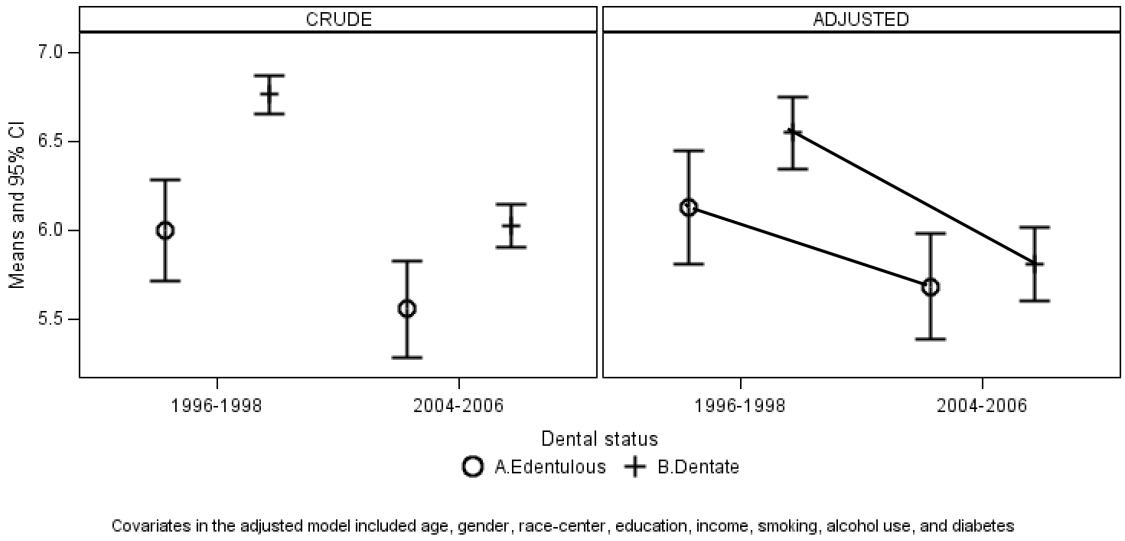


Figure 5-1. Crude and adjusted means with 95% confidence intervals for the Delayed Word Recall test scores at baseline and follow-up comparing edentulous with dentate participants (n = 911)

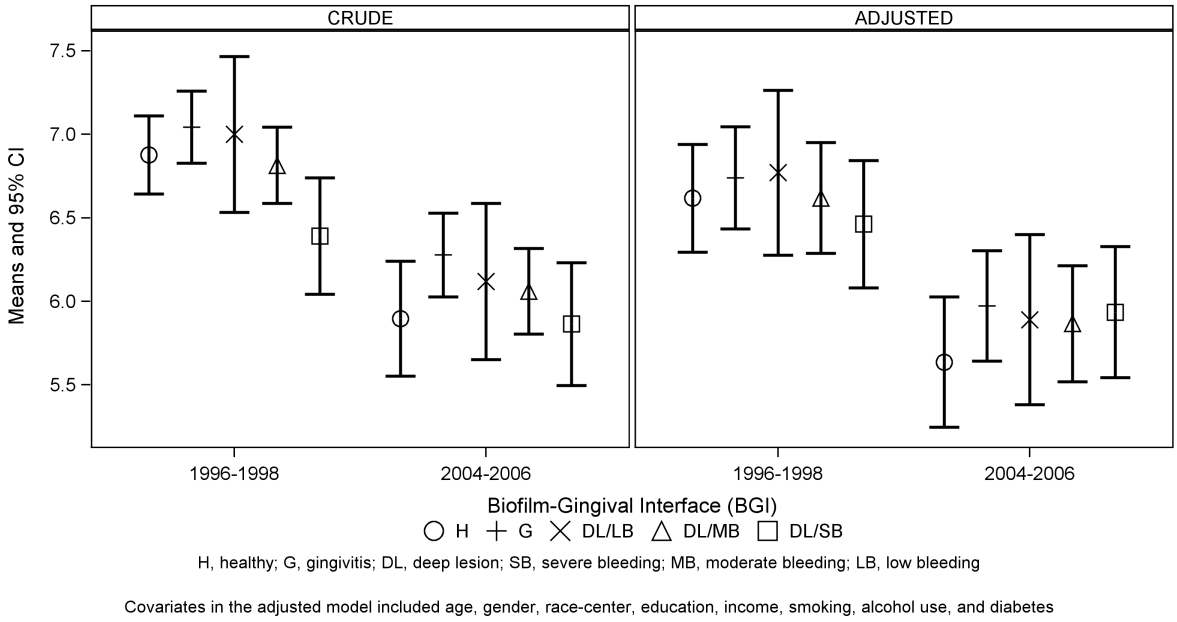


Figure 5-2. Crude and adjusted means and 95% confidences intervals for the Delayed Word Recall test scores at baseline and follow-up in relation to five levels of periodontal conditions (n = 558)

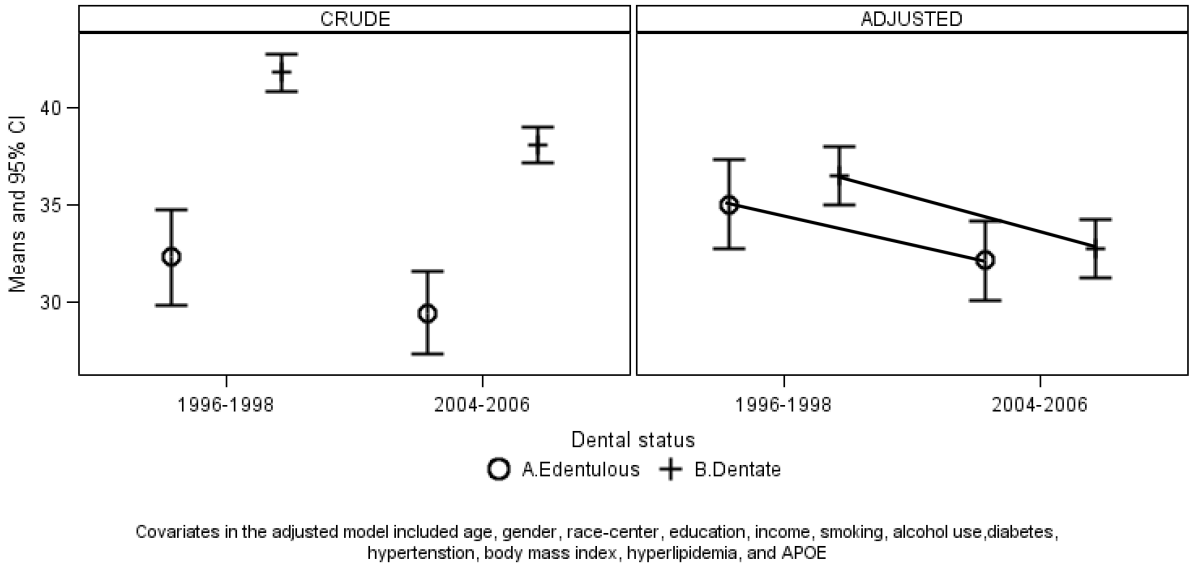


Figure 5-3. Crude and adjusted means and 95% confidences intervals for the Digit Symbol Substitution test scores at baseline and follow-up comparing edentulous with dentate participants (n = 911)

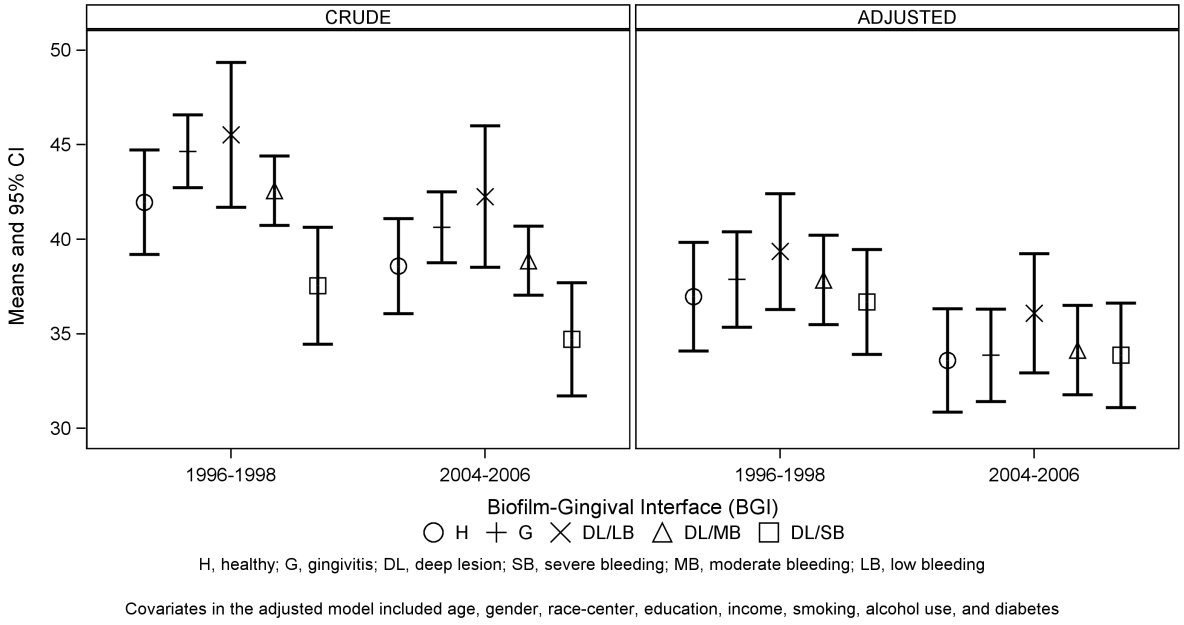


Figure 5-4. Crude and adjusted means and 95% confidences intervals of Digit Symbol Substitution test scores at baseline and follow up in relation to five levels of periodontal conditions (n = 558)

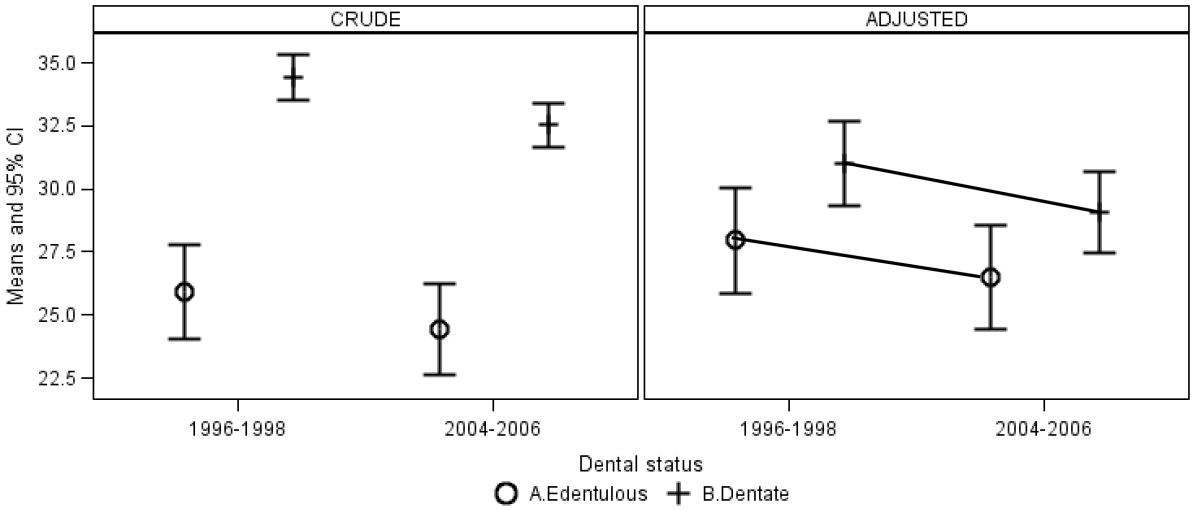


Figure 5-5. Crude and adjusted means and 95% confidences intervals of Word Fluency test scores at baseline and follow up comparing edentulous with dentate participants (n = 911)

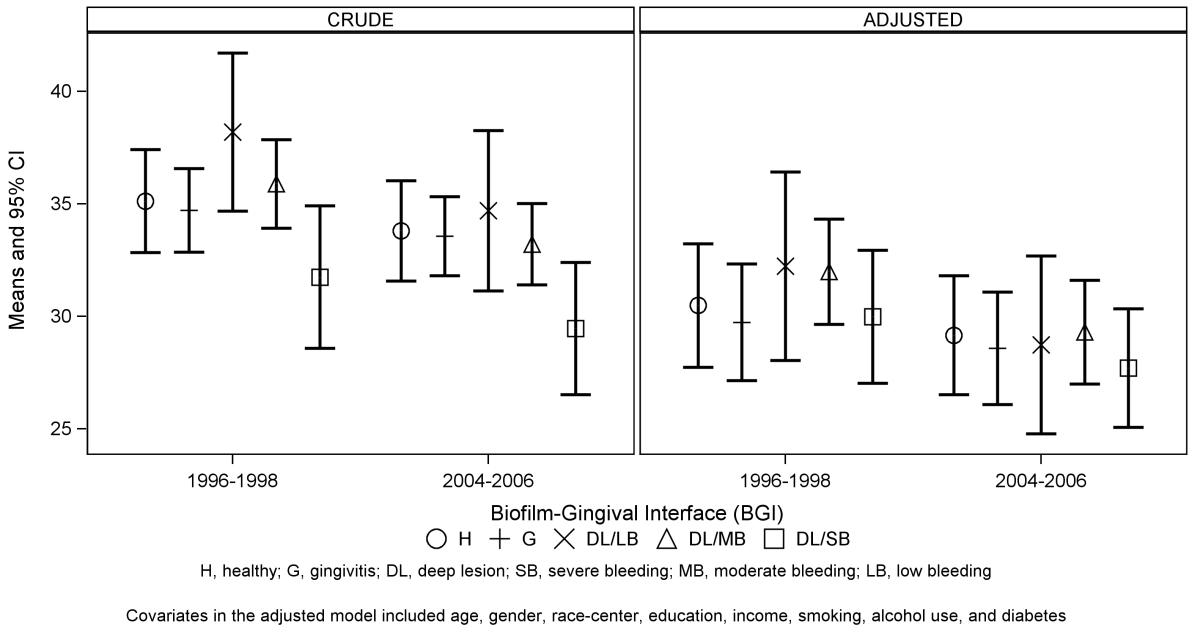


Figure 5-6. Crude and adjusted means and 95% confidences intervals of Word Fluency test scores at baseline and follow up in relation to five levels of periodontal conditions (n = 558)

J. Supplemental materials

Covariates

Diabetic status was determined by fasting plasma glucose ≥ 126 mg/dL, non-fasting plasma glucose ≥ 200 mg/dL, self-reported- history of physician-diagnosed diabetes or current medication for diabetes. Hypertension was defined as a previous diagnosis of hypertension, taking hypertensive medication, or having a current systolic blood pressure of 140 mmHg or higher or a diastolic blood pressure 90 mmHg. Stroke was defined as a self-reported history of physician-diagnosed stroke or stroke validated by an ARIC clinician through a review of medical records. Coronary heart disease was defined as adjudicated myocardial infraction on the electrocardiogram at baseline, or prior self-reported history of myocardial infarction, coronary artery bypass surgery or angioplasty.

Additional analyses and results

Effect measure modification

We also tested whether diabetes and APOE ε4 were the effect measure modifiers by examining likelihood ratio for type 3 analysis of the interaction terms between presumed effect modifiers, oral health measures, and time. If the *p-value* is less than 0.10, the interaction term is included in the regression model. In this study sample, no effect measure modification by diabetes and APOE ε4 was observed.

Secondary analyses

The present longitudinal analysis included a substudy sample (~10%) of our previous cross-sectional study (Study #1), and differences in study characteristics between the cross-sectional and longitudinal study participants were evident. Therefore, the cross-sectional analysis, examining the association between oral health measures and all three cognitive tests at Visit 4, was repeated in the present study sample.

Complete tooth loss was significantly associated with only low DWR and WF scores. Periodontal disease and the number of teeth were not associated with all Visit 4 cognitive scores (Table 5-4).These results were consistent with the longitudinal analysis using GEE models. However, these cross-sectional findings differed from our prior cross-sectional study, which comprised of all available Visit 4 ARIC cohort members. The previous study found that complete tooth loss was related with all three cognitive performance. Furthermore, the associations of periodontal disease and the number of teeth with DSS and WF scores were observed.

Differences in socio-demographic backgrounds as well as cognitive function at baseline between participants from Forsyth County and Jackson were also noticeable. Therefore, we performed a stratified analysis by study sites to investigate whether the associations between oral health measures and cognitive performance differed across the study sties. The sites-specific estimates were shown in Table 5-6. Overall, the effect of oral health measures on cognitive profile was more pronounced in Forsyth County than that in Jackson.

K. Supplemental Tables

Table 5-4. Regression coefficients for the associations between oral health measures and Visit 4 cognitive scores: A cross-sectional analysis

		Delayed word recall		Digit symbol substitution		Word fluency	
	n	b (SE)	*P-value*	b (SE)	*P-value*	b (SE)	*P-value*
Dental status[1,2]	911						
Edentulous		-0.44 (0.15)	**0.0026**	-1.40 (1.00)	0.1136	-3.16 (1.12)	**0.0049**
Dentate		Ref		Ref		Ref	
Periodontal disease[1]	558						
Had periodontal pockets							
BOP > 50%		-0.22 (0.21)	0.6788	-0.20 (1.44)	0.6173	-0.058 (1.76)	0.2981
BOP 10- ≤ 50%		0.0069 (0.17)		0.89 (1.14)		1.82 (1.40)	
BOP < 10%		0.13 (0.25)		2.33 (1.69)		1.74 (2.06)	
No periodontal pockets							
BOP ≥ 10%		0.11 (0.17)		0.76 (1.17)		-0.65 (1.44)	
BOP < 10%		Ref		Ref		Ref	
Number of teeth[1]	558						
1-tooth increase		0.007 (0.009)	0.4261	0.031(0.06)	0.5961	0.018 (0.07)	0.7997

b , regression coefficient; SE, standard error; BGI; Biofilm-Gingival Interface
BGI classified periodontal disease based on probing pocket depth (PPD) and the extent of bleeding on probing (BOP). Five levels of BGI were the followings: BGI-DL/SB (deep lesion/severe bleeding), BGI-DL/MB (deep lesion/moderate bleeding), BGI-DL/LB (deep lesion/low bleeding), BGI-G (gingivitis), and BGI-H (healthy).
[1]Adjusting for age, gender, race-center, income, smoking, alcohol use, and diabetes
[2]Digit symbol substitution: Adjusting for age, gender, race-center, education, income, smoking, alcohol use, diabetes, hypertension, hyperlipidemia, body mass index, and APOE ε4

Table 5-5. Comparison of cognitive scores at baseline and follow-up of participants who were from Forsyth County NC and Jackson MS

Cognitive tests	Study sites				*P-value*	All (n = 911)	
	Forsyth County n = 518 (56.9%)		Jackson n = 393 (43.1%)				
	Mean	SD	Mean	SD		Mean	SD
Delayed word recall							
1996-1998	7.0	1.4	6.2	1.6	<0.0001	6.7	1.6
2004-2006	6.3	1.6	5.5	1.7	<0.0001	6.0	1.7
Change	-0.7	1.7	-0.7	1.8	0.7733	-0.7	1.7
Digit symbol substitution							
1996-1998	47.1	11.4	31.9	12.4	<0.0001	40.5	14.0
2004-2006	43.1	10.9	28.7	11.5	<0.0001	36.9	13.2
Change	-4.0	6.7	-3.2	8.7	0.0905	-3.6	7.6
Word fluency							
1996-1998	36.0	12.0	29.6	13.0	<0.0001	33.3	12.9
2004-2660	34.8	11.6	27.0	11.7	<0.0001	31.4	12.3
Change	-1.2	7.4	-2.7	7.9	0.0034	-1.9	7.6

Change = (Scores at 2004-2006 – Scores at 1996-1998)

Table 5-6. Study site-specific estimates for associations between time, dental status, and their on three measures of cognitive function

	Forsyth County (n = 518)			Jackson (n = 393)			All (n = 911)		
	b	SE	*P-value*	b	SE	*P-value*	b	SE	*P-value*
Delayed word recall[1]									
Time (F/U vs. Baseline)	-0.74	0.08	**0.0001**	-0.73	0.10	**<0.0001**	-0.74	0.061	**<0.0001**
Edentulous	-0.68	0.24	**0.0250**	-0.29	0.21	0.3028	-0.42	0.16	**0.0357**
Edentulous x Time	0.37	0.25	0.1500	0.25	0.23	0.2827	0.29	0.17	**0.0855**
Digit symbol substitution[2]									
Time (F/U vs. Baseline)	-4.18	0.30	**<0.0001**	-3.14	0.45	**<0.0001**	-3.76	0.26	**<0.0001**
Edentulous	-3.75	1.73	0.0638	-0.13	1.38	0.8908	-1.47	1.08	0.2438
Edentulous x Time	1.86	1.24	0.1416	-0.04	1.30	0.9729	0.89	0.93	0.3389
Word fluency[1]									
Time (F/U vs. Baseline)	-1.28	0.35	0.0543	-2.86	0.46	**<0.0001**	-1.91	0.28	**<0.0001**
Edentulous	-4.74	1.40	**0.0045**	-2.34	1.20	0.0677	-3.03	0.92	**0.0014**
Edentulous x Time	0.93	0.82	0.2645	0.79	0.85	0.3524	0.46	0.60	0.4512

b , regression coefficient; SE, standard error

[1]Adjusting for age, gender, education, income, smoking, alcohol use, and diabetes

[2]Adjusting for age, gender, education, income, smoking,, alcohol use, and diabetes, hypertension, hyperlipidemia, body mass index, and APOE ε4

Table 5-7. Regression coefficients for the effects of time, oral health measures, and their interaction on three cognitive scores, excluding subjects with stroke at baseline

	n	Delayed word recall		Digit symbol substitution		Word fluency	
		b (SE)	*P-value*	b (SE)	*P-value*	b (SE)	*P-value*
Dental status[1,2]	894						
Time (F/U *vs.* Baseline)		-0.74 (0.06)	**<0.0001**	-3.74 (0.26)	**<0.0001**	-1.83 (0.28)	**< 0.0001**
Edentulous		-0.45 (0.16)	**0.0261**	-1.35 (1.08)	0.2810	-3.32 (0.93)	**0.0004**
Edentulous x Time		0.31 (0.17)	**0.0719**	0.83 (0.95)	0.3859	0.42 (0.61)	0.5000
Periodontal disease[1]	550						
Time (F/U *vs*. Baseline)		-1.01 (0.18)	**<0.0001**	-3.59 (0.83)	**<0.0001**	-1.12 (0.81)	**<0.0001**
BGI-DL/SB		-0.20 (0.21)	0.6331	-0.14 (1.56)	0.3609	-0.31 (1.78)	0.5021
BGI-DL/MB		0.0029 (0.16)		1.28 (1.17)		2.00 (1.43)	
BGI-DL/LB		0.12 (0.24)		2.62 (1.56)		2.12 (2.14)	
BGI-G		0.092 (0.16)		1.24 (1.21)		-0.33 (1.39)	
BGI-DL/SB x Time		0.48 (0.28)	0.4935	0.77 (1.13)	0.7867	-1.17 (1.18)	0.2133
BGI-DL/MB x Time		0.27 (0.22)		-0.086 (0.95)		-1.47 (1.01)	
GI-DL/LB x Time		0.13 (0.29)		0.33 (1.32)		-2.38 (1.46)	
BGI-G x Time		0.25 (0.22)		-0.41 (0.99)		-0.024 (0.99)	
Number of teeth[1]	550						
Time (F/U *vs.* Baseline)		-0.68 (0.23)	**0.0047**	-3.24 (0.88)	**0.0003**	-2.55 (0.92)	**0.0060**
1-tooth increase		0.0017 (0.0086)	0.8458	0.052 (0.056)	0.3471	-0.0085 (0.074)	0.9098
1-tooth increase x Time		-0.0046 (0.01)	0.6581	-0.018 (0.039)	0.6507	0.030 (0.043)	0.4870

b , regression coefficient; SE, standard error; BGI, Biofilm -Gingival Interface
BGI classified periodontal disease based on probing pocket depth (PPD) and the extent of bleeding on probing (BOP).
BGI-DL/SB (deep lesion/severe bleeding), BGI-DL/MB (deep lesion/moderate bleeding), BGI-DL/LB (deep lesion/low bleeding), BGI-G (gingivitis), and BGI-H (healthy).
[1]Adjusting for age, gender, race-center, education, income, smoking, alcohol use, and diabetes
[2]Digit symbol substitution: Adjusting for age, gender, race-center, education, income, smoking, alcohol use, diabetes, hypertension, hyperlipidemia, body mass index, and APOE ε4

Table 5-8. Regression coefficients for effects oral health measures on cognitive function changes: Change score models

	n	Delayed word recall		Digit symbol substitution		Word fluency	
		b (SE)	*P-value*	b (SE)	*P-value*	b (SE)	*P-value*
Dental status[1,2]	991						
Edentulous		0.33 (0.17)	**0.0536**	0.75 (0.77)	0.3297	0.71 (0.77)	0.3571
Dentate		Ref		Ref		Ref	
Periodontal disease[1]	558						
Had periodontal pockets							
BOP ≥ 50%		0.50 (0.26)	0.4392	0.39 (1.06)	0.9255	-1.83 (1.18)	0.0603
BOP 10- <50%		0.22 (0.21)		-0.35 (0.84)		-2.00 (0.93)	
BOP < 10%		0.15 (0.31)		0.21 (1.23)		-2.17 (1.37)	
No periodontal pockets							
BOP ≥ 10%		0.24 (0.21)		-0.34 (0.86)		-0.021 (0.96)	
BOP < 10%		Ref		Ref		Ref	
Number of teeth[1]	558						0.7364
1-tooth increase		-0.013 (0.011)	0.2291	0.024 (0.043)	0.5732	-0.016 (0.049)	

b, regression coefficient; SE, standard error; BGI, Biofilm -Gingival Interface
Change scores = (Scores at follow-up) – (Scores at baseline)
BGI classified periodontal disease based on probing pocket depth (PPD) and the extent of bleeding on probing (BOP).
BGI-DL/SB (deep lesion/severe bleeding), BGI-DL/MB (deep lesion/moderate bleeding), BGI-DL/LB (deep lesion/low bleeding), BGI-G (gingivitis), and BGI-H (healthy).
[1]Adjusting for age, gender, race-center, education, income, smoking, alcohol use, and diabetes
[2]Digit symbol substitution: Adjusting for age, gender, race-center, education, income, smoking, alcohol use, diabetes, hypertension, hyperlipidemia, body mass index, and APOE ε4

Table 5-9**.** Baseline characteristics and repeated measures (1996-1998 and 2004-2006) of the three cognitive test scores (n = 911)

Characteristics	Col %	Delayed word recall		Digit symbol substitution		Word fluency	
		b (SE)	*P-value*	b (SE)	*P-value*	b (SE)	*P-value*
Age categories							
≥ 65	48.3	-0.44 (0.14)	0.0017	-5.05 (1.43)	0.0005	-2.54 (1.28)	0.1457
60-64	39.6	-0.19 (0.14)		-2.48 (1.46)		-2.03 (1.28)	
51-59	12.1	Ref		Ref		Ref	
Gender							
Male	38.6	-0.48 (0.094)	<0.0001	-3.13 (0.89)	0.0005	-1.01 (0.82)	0.2244
Female	61.4	Ref		Ref		Ref	
Race							
African American	48.5	-0.75 (0.088)	<0.0001	-14.48 (0.72)	<0.0001	-6.7 (0.76)	<0.0001
White	51.5	Ref		Ref		Ref	
Study sites							
Forsyth	56.9	0.79 (0.088)	<0.0001	14.76 (0.73)	<0.0001	7.10 (0.77)	<0.0001
Jackson	43.1	Ref		Ref		Ref	
Education							
Less than high school	20.0	-0.87 (0.12)	<0.0001	-17.35 (0.95)	<0.0001	-15.53 (0.87)	<0.0001
High school completion	34.8	-0.21 (0.098)		-3.84 (0.86)		6.66 (0.79)	
Post-secondary education	45.2	Ref		Ref		Ref	
Income							
Refused	2.4	0.024 (0.26)	<0.0001	-7.13 (2.93)	<0.0001	-5.15 (2.36)	<0.0001
<25000	38.5	-0.64 (0.11)		-14.37 (0.95)		-10.94 (0.91)	
25-<50000	31.4	-0.26 (0.12)		-5.58 (0.97)		-3.53 (0.96)	
50000 or more	27.7	Ref		Ref		Ref	
Cigarette use							
Current	13.0	-0.095 (0.13)	0.6417	-3.26 (1.26)	0.0232	-1.40 (1.23)	<0.2513
Former	38.9	-0.079 (0.10)		0.17 (0.94)		0.73 (0.86)	
Never	48.1	Ref		Ref		Ref	
Alcohol use							
Current	35.8	0.34 (0.11)	0.0002	7.86 (1.04)	<0.0001	6.08 (0.96)	<0.001
Former	34.0	-0.092 (0.11)		0.29 (1.02)		0.42 (0.94)	
Never	30.2	Ref		Ref		Ref	
Diabetes mellitus							
Yes	16.7	-0.30 (0.13)	0.0193	-6.12 (1.15)	<0.0001	-4.82 (0.97)	<0.0001
No	83.3	Ref		Ref		Ref	
Hypertension							
Yes	48.4	-0.36 (0.09)	<0.0001	-6.68 (0.84)	<0.0001	-3.84 (0.78)	<0.0001
No	51.6	Ref		Ref		Ref	
CHD							
Yes	4.6	-0.17 (0.24)	0.4998	-0.048 (1.73)	0.9778	0.76 (1.79)	0.6727
No	93.6	Ref		Ref		Ref	
Missing	1.8						
Stroke							
Yes	1.9	-0.98 (0.31)	0.0120	-5.59 (3.08)	0.0956	-1.21 (3.09)	0.6969
No	98.1	Ref		Ref		Ref	
Missing	0.11						
Hyperlipidemia							
Yes	34.8	0.13 (0.095)	0.1738	0.18 (0.93)	0.8454	-0.32 (0.81)	0.6965
No	65.2	Ref		Ref		Ref	
BMI (kg/m^2)							
≥ 30	31.5	-0.32 (0.82)	0.6965	-0.24 (0.097)	0.0139	-5.54 (0.89)	<0.0001
< 30	68.5	Ref		Ref		Ref	

APOE ε4							
Yes	30.6	-0.21 (0.10)	0.0427	-2.97 (0.96)	0.0023	-1.20 (0.87)	0.1718
No	69.4	Ref		Ref		Ref	
Oral health conditions							
Edentulous							
Yes	13.8	-0.62 (0.13)	<0.0001	-9.08 (1.17)	<0.0001	-8.30 (0.99)	<0.0001
No	86.2	Ref		Ref		Ref	
Number of teeth[1]							
1-24	57.5	-0.36 (0.11)	0.0016	-7.28 (1.02)	<0.0001	-4.73 (1.00)	<0.0001
≥ 25	42.5	Ref		Ref		Ref	
Periodontal disease[1]							
Had periodontal pockets							
BOP > 50%	13.3	-0.26 (0.19)	0.0577	-4.14 (1.98)	0.0047	-3.86 (1.85)	0.1249
BOP 10- ≤ 50%	31.9	0.051 (0.16)		0.45 (1.57)		0.083 (1.42)	
BOP < 10%	7.5	0.17 (0.24)		3.62 (2.26)		1.98 (2.01)	
No periodontal pockets							
BOP ≥ 10%	28.3	0.27 (0.16)		2.37 (1.58)		-0.32 (1.39)	
BOP < 10%	19.0	Ref		Ref		Ref	

n, total number of study group; CHD, coronary heart disease; BMI, body mass index; BGI, Biofilm-Gingival Interface
BGI classified periodontal disease based on probing pocket depth (PPD) and the extent of bleeding on probing (BOP).
[1]Only among dentate participants who received periodontal examination (n = 558)

Table 5-10. Regression coefficients for effects of time, dental status, and their interaction (n = 911)

Models	Delayed word recall			Digit symbol substitution			Word fluency		
	b	SE	*P-value*	b	SE	*P-value*	b	SE	*P-value*
Crude									
Time (F/U *vs*. Baseline)	-0.74	0.06	**<0.0001**	-3.76	0.26	**<0.0001**	-1.91	0.28	**<0.0001**
Edentulous	-0.76	0.15	**<0.0001**	-9.52	1.34	**<0.0001**	-8.53	1.05	**<0.0001**
Edentulous x Time	0.29	0.17	0.0855	0.89	0.93	0.3389	0.46	0.60	0.4512
Model A[1]									
Time (F/U *vs*. Baseline)	-0.74	0.06	**<0.0001**	-3.76	0.26	**<0.0001**	-1.91	0.28	**<0.0001**
Edentulous	-0.44	0.16	**0.0241**	-2.24	1.10	0.4601	-3.20	0.92	**0.0007**
Edentulous x Time	0.29	0.17	0.0855	0.89	0.93	0.3389	0.46	0.60	0.4512
Model B[2]									
Time (F/U *vs*. Baseline)	-0.74	0.06	**<0.0001**	-3.76	0.26	**<0.0001**	-1.91	0.28	**<0.0001**
Edentulous	-0.42	0.16	**0.0357**	-1.74	1.08	0.1438	-3.03	0.92	**0.0014**
Edentulous x Time	0.29	0.17	0.0828	0.89	0.93	0.3389	0.46	0.60	0.4512
Model C[3]									
Time (F/U *vs*. Baseline)	-0.74	0.06	**<0.0001**	-3.76	0.26	**<0.0001**	-1.91	0.28	**<0.0001**
Edentulous	-0.41	0.16	**0.0487**	-1.47	1.08	0.2438	-2.94	0.93	**0.0021**
Edentulous x Time	0.29	0.17	0.0828	0.89	0.93	0.3389	0.46	0.60	0.4512

b, regression coefficient; SE, standard error
[1]Adjusting for age, gender, race-center, education, and income
[2]Adjusting for covariates in model A, smoking,, alcohol use, and diabetes (minimally sufficient adjustment set)
[3]Adjusting for covariates in model B, hypertension, hyperlipidemia, body mass index, and APOE ε4

Table 5-11. Stratum-specific estimates for associations between dental status and cognitive scores (n = 911)

Models	Visit	Delayed word recall		Digit symbol substitution		Word fluency	
		b	SE	b	SE	b	SE
Model B[1]							
Edentulous	1996-1998	-0.42	0.16	-1.74	1.08	-3.03	0.92
Edentulous	2004-2006	-0.13	0.15	-0.85	0.90	-2.58	0.87
Model C[2]							
Edentulous	1996-1998	-0.41	0.16	-1.47	1.08	-2.94	0.93
Edentulous	2004-2006	-0.11	0.15	-0.58	0.90	-2.49	0.88
Change %[3]							
	1996-1998	-1.7		-27.3		-9.0	
	2004-2006	-1.7		-27.3		-9.0	

b, regression coefficient; SE, standard error

[1]Adjusting for age, gender, race-center, education, income, smoking, alcohol use, and diabetes (a minimally sufficient adjustment set)

[2]Adjusting for covariates in model B, hypertension, hyperlipidemia, body mass index, and APOE ε4

[3]Changes in estimates = $(b_{\text{model B}} - b_{\text{model C}}) \times 100$

Table 5-12. Regression coefficients for effects of time, periodontal disease, and their interaction on cognitive scores (n = 558)

Models	Delayed word recall			Digit symbol substitution			Word fluency		
	b	SE	*P-value*	b	SE	*P-value*	b	SE	*P-value*
Crude									
Time (F/U *vs*. Baseline)	-0.98	0.18	**<0.0001**	-3.38	0.84	**<0.0001**	-1.32	0.80	**<0.0001**
BGI-DL/SB	-0.49	0.21	0.0577	-4.42	2.12	**0.0047**	-3.38	2.00	0.1249
BGI-DL/MB	-0.06	0.17		0.62	1.70		0.77	1.54	
BGI-DL/LB	0.12	0.27		3.56	2.41		3.07	2.14	
BGI-G	0.17	0.16		2.69	1.72		-0.41	1.50	
BGI-DL/SB x Time	0.45	0.27	0.5655	0.55	1.14	0.7636	-0.96	1.17	0.2173
BGI-DL/MB x Time	0.23	0.22		-0.33	0.96		-1.36	1.00	
BGI-DL/LB x Time	0.10	0.29		0.12	1.32		-2.18	1.45	
BGI-G x Time	0.22	0.22		-0.64	0.99		0.17	0.98	
Model A[1]									
Time (F/U *vs*. Baseline)	-0.98	0.18	**<0.0001**	-3.38	0.84	**<0.0001**	-1.32	0.80	**<0.0001**
BGI-DL/SB[1]	-0.17	0.21	0.5895	-0.42	1.60	0.6229	-0.63	1.79	0.6179
BGI-DL/MB	0.14	0.24		2.20	1.64		1.67	2.15	
BGI-DL/LB	-0.01	0.16		0.65	1.20		1.28	1.42	
BGI-G	0.12	0.16		0.73	1.23		-1.00	1.37	
BGI-DL/SB x Time	0.45	0.27	0.5655	0.55	1.14	0.7673	-0.96	1.17	0.2173
BGI-DL/MB x Time	0.10	0.29		0.12	1.32		-2.18	1.45	
BGI-DL/LB x Time	0.23	0.22		-0.33	0.96		-1.36	1.00	
BGI-G x Time	0.22	0.22		-0.64	0.99		0.17	0.98	
Model B[2]									
Time (F/U *vs*. Baseline)	-0.98	0.18	**<0.001**	-3.38	0.84	**<0.0001**	-1.32	0.80	**<0.0001**
BGI-DL/SB[1]	-0.16	0.21	0.6040	-0.28	1.56	0.5165	-0.50	1.79	0.6225
BGI-DL/MB	0.0015	0.16		0.88	1.18		1.50	1.44	
BGI-DL/LB	0.15	0.24		2.38	1.56		1.75	2.15	
BGI-G	0.12	0.16		0.91	1.22		-0.75	1.40	
BGI-DL/SB x Time	0.45	0.27	0.5655	0.55	1.14	0.7636	-0.96	1.17	0.2173
BGI-DL/MB x Time	0.23	0.22		-0.33	0.96		-1.36	1.00	
BGI-DL/LB x Time	0.10	0.29		0.12	1.32		-2.18	1.45	
BGI-G x Time	0.22	0.22		-0.64	0.99		0.17	0.98	
Model C[3]									
Time (F/U *vs*. Baseline)	-0.98	0.18	**<0.0001**	-3.38	0.84	**<0.0001**	-1.32	0.80	**<0.0001**
BGI-DL/SB[1]	-0.14	0.21	0.6173	-0.31	1.55	0.5316	-0.40	1.79	0.6577
BGI-DL/MB	0.0039	0.16		0.92	1.17		1.51	1.43	
BGI-DL/LB	0.14	0.24		2.32	1.56		1.75	2.17	
BGI-G	0.12	0.16		0.97	1.21		-0.70	1.40	
BGI-DL/SB x Time	0.45	0.27	0.5655	0.55	1.14	0.7636	-0.96	1.17	0.2173
BGI-DL/MB x Time	0.23	0.22		-0.33	0.96		-1.36	1.00	
BGI-DL/LB x Time	0.10	0.29		0.12	1.32		-2.18	1.45	
BGI-G x Time	0.22	0.22		-0.64	0.99		0.17	0.98	

b, regression coefficient; SE, standard error; BGI, Biofilm-Gingival Interface
[1]Adjusting for age, gender, race-center, education, and income
[2]Adjusting for covariates in model A, smoking,, alcohol use, and diabetes (a minimally sufficient adjustment set)
[3]Adjusting for covariates in model B, hypertension, hyperlipidemia, body mass index, and APOE ε4

Table 5-13. Stratum-specific estimates for associations between periodontal disease and cognitive scores (n = 558)

Models	Visit	Delayed word recall		Digit symbol substitution		Word fluency	
		b	SE	b	SE	b	SE
Model B[1]							
BGI-DL/SB	1996-1998	-0.16	0.21	-0.28	1.56	-0.50	1.79
BGI-DL/MB		0.002	0.16	0.88	1.18	1.50	1.44
BGI-DL/LB		0.15	0.24	2.38	1.56	1.75	2.15
BGI-G		0.15	0.16	2.38	1.22	1.75	1.40
BGI-DL/SB	2004-2006	0.30	0.24	0.28	1.55	-1.46	1.58
GI-DL/MB		0.23	0.21	0.55	1.14	0.14	1.33
BGI-DL/LB		0.25	0.28	2.50	1.58	-0.43	2.01
BGI-G		0.34	0.21	0.27	1.15	-0.58	1.29
Model C[1]							
BGI-DL/SB	1996-1998	-0.14	0.21	-0.31	1.55	-0.40	1.79
BGI-DL/MB		0.004	0.16	0.92	1.17	1.51	1.43
BGI-DL/LB		0.14	0.24	2.32	1.56	1.75	2.17
BGI-G		0.14	0.16	2.32	1.21	1.75	1.40
BGI-DL/SB	2004-2006	0.32	0.24	0.25	1.53	-1.37	1.58
BGI-DL/MB		0.23	0.21	0.59	1.13	0.15	1.33
BGI-DL/LB		0.24	0.28	2.43	1.55	-0.43	2.02
BGI-G		0.34	0.21	0.33	1.14	-0.53	1.35
Change %[3]							
BGI-DL/SB	1996-1998	-1.6		3.0		-9.3	
BGI-DL/MB		-0.2		-4.1		-0.7	
BGI-DL/LB		0.9		6.4		-0.1	
BGI-G		0.9		6.4		-0.1	
BGI-DL/SB	2004-2006	-1.6		3.0		-9.3	
BGI-DL/MB		-0.2		-4.1		-0.7	
BGI-DL/LB		0.9		6.4		-0.1	
BGI-G		-0.03		-5.8		-5.0	

b, regression coefficient; SE, standard error; BGI, Biofilm-Gingival Interface

BGI classified periodontal disease based on probing pocket depth (PPD) and the extent of bleeding on probing (BOP).

BGI-DL/SB (deep lesion/severe bleeding), BGI-DL/MB (deep lesion/moderate bleeding), BGI-DL/LB (deep lesion/low bleeding), BGI-G (gingivitis), and BGI-H (healthy).

[1]Adjusting for age, gender, race-center, education, income, smoking, alcohol use, and diabetes (a minimally sufficient adjustment set)

[2]Adjusting for covariates in model B, hypertension, hyperlipidemia, body mass index, and APOE ε4

[3]Changes in estimates = $(b_{\text{model B}} - b_{\text{model C}}) \times 100$

Table 5-14. Regression coefficients for effects of time, number of teeth, and their interaction on cognitive scores (n = 558)

Models	Delayed word recall			Digit symbol substitution			Word fluency		
	b	SE	*P-value*	b	SE	*P-value*	b	SE	*P-value*
Crude									
Time (F/U *vs*. Baseline)	-0.67	0.23	**0.0048**	-3.38	0.86	**<0.0001**	-2.72	0.90	**0.0028**
1-tooth increase	0.028	0.008	**0.0011**	0.55	0.073	**<0.0001**	0.30	0.075	**<0.0001**
1-tooth increase x Time	-0.0053	0.010	0.6109	-0.0094	0.039	0.8071	0.035	0.042	0.4100
Model A[1]									
Time (F/U *vs*. Baseline)	-0.67	0.23	**0.0048**	-3.38	0.86	**0.0001**	-2.72	0.90	**0.0028**
1-tooth increase	0.0052	0.0086	0.5455	0.072	0.058	0.2168	0.017	0.073	0.8209
1-tooth increase x Time	-0.0053	0.010	0.6109	-0.0094	0.039	0.8071	0.035	0.042	0.4100
Model B[2]									
Time (F/U *vs*. Baseline)	-0.67	0.23	**0.0048**	-3.38	0.86	**0.0001**	-2.72	0.90	**0.0028**
1-tooth increase	0.0031	0.0086	0.7129	0.046	0.056	0.3959	-0.0071	0.073	0.9233
1-tooth increase x Time	-0.0053	0.010	0.6109	-0.0094	0.039	0.8071	0.035	0.042	0.4100
Model C[3]									
Time (F/U *vs*. Baseline)	-0.67	0.23	**0.0048**	-3.38	0.86	**0.0001**	-2.72	0.90	**0.0028**
1-tooth increase	0.0027	0.0087	0.7568	0.040	0.057	0.4825	-0.017	0.073	0.8157
1-tooth increase x Time	-0.0053	0.0104	0.6109	-0.0094	0.039	0.8071	0.035	0.042	0.4100

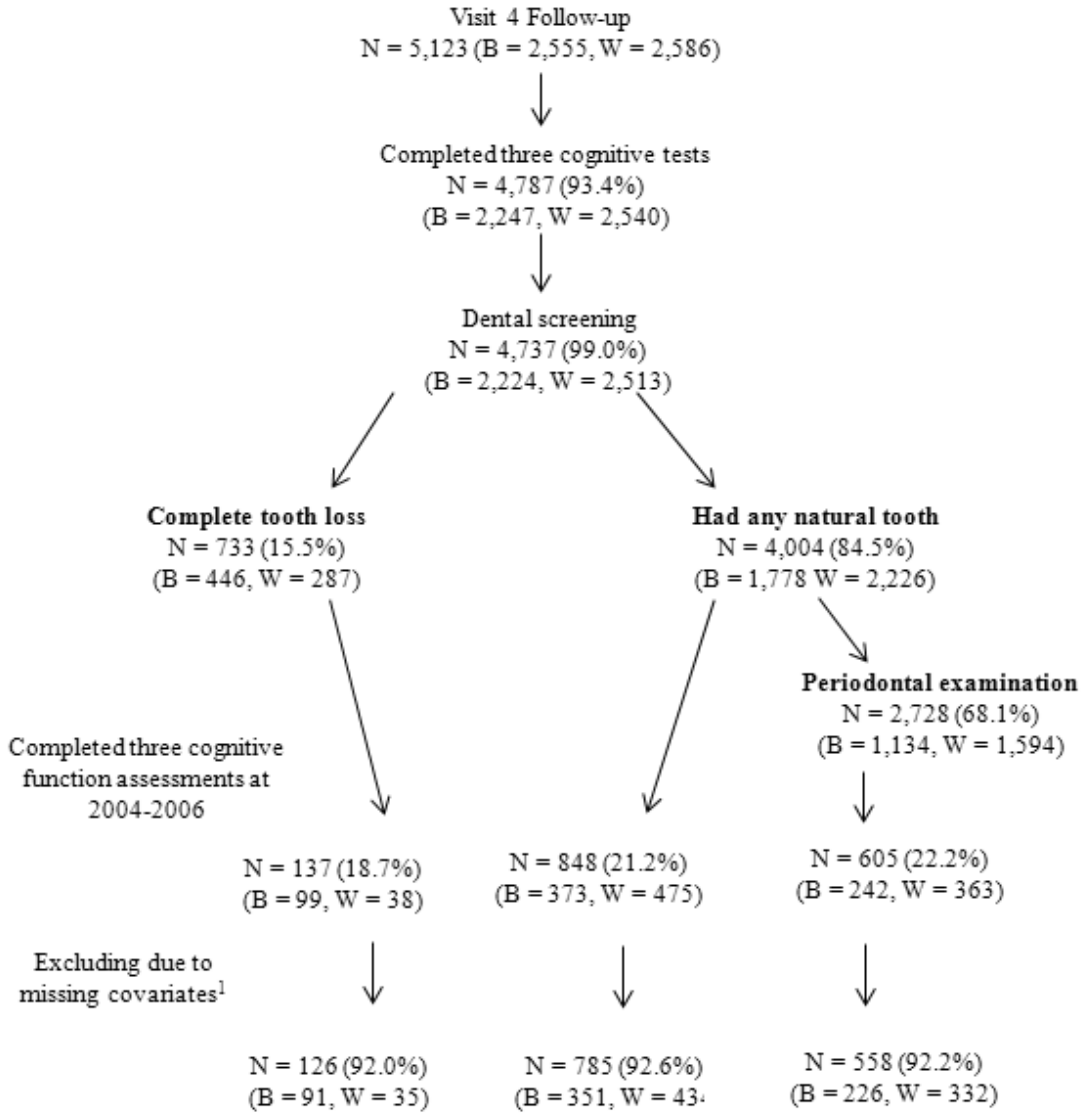
b, coefficient; SE, standard error
[1]Adjusting for age, gender, race-center, education, and income
[2]Adjusting for covariates in model A, smoking, alcohol use, and diabetes (a minimally sufficient adjustment set)
[3]Adjusting for covariates in model B, hypertension, hyperlipidemia, body mass index, and APOE ε4

Table 5-15. Stratum-specific estimates for the association between number of teeth and cognitive scores (n = 558)

Model	Visit	Delayed word recall		Digit symbol substitution		Word fluency	
		b	SE	b	SE	b	SE
Model B[1]							
1-tooth increase	1996-1998	0.0032	0.0086	0.048	0.057	-0.0071	0.073
1-tooth increase	2004-2006	-0.0021	0.0095	0.039	0.055	0.028	0.066
Model C[2]							
1-tooth increase	1996-1998	0.0027	0.0087	0.040	0.057	-0.017	0.073
1-tooth increase	2004-2006	-0.0026	0.0095	0.031	0.055	0.018	0.066
Change %[3]							
	1996-1998	0.05		0.8		1.0	
	2004-2006	0.05		0.8		1.0	

b, regression coefficient; SE, standard error
[1]Adjusting for age, gender, race-center, education, income, smoking, alcohol use, and diabetes (a minimally sufficient adjustment set)
[2]Adjusting for covariates in model B, hypertension, hyperlipidemia, body mass index, and APOE ε4
[3]Changes in estimates = $(b_{\text{model B}} - b_{\text{model C}}) \times 100$

L. Supplemental figures



[1]Covariates: age, race, gender, study sites, education, income, smoking, alcohol use, body mass index, hyperlipidemia, diabetes, and APOE ε4.

Figure 5-7. Flow chart of ARIC participants who completed two cognitive function assessments (1996-1998 and 2004-2006) and participated in the Dental ARIC Study

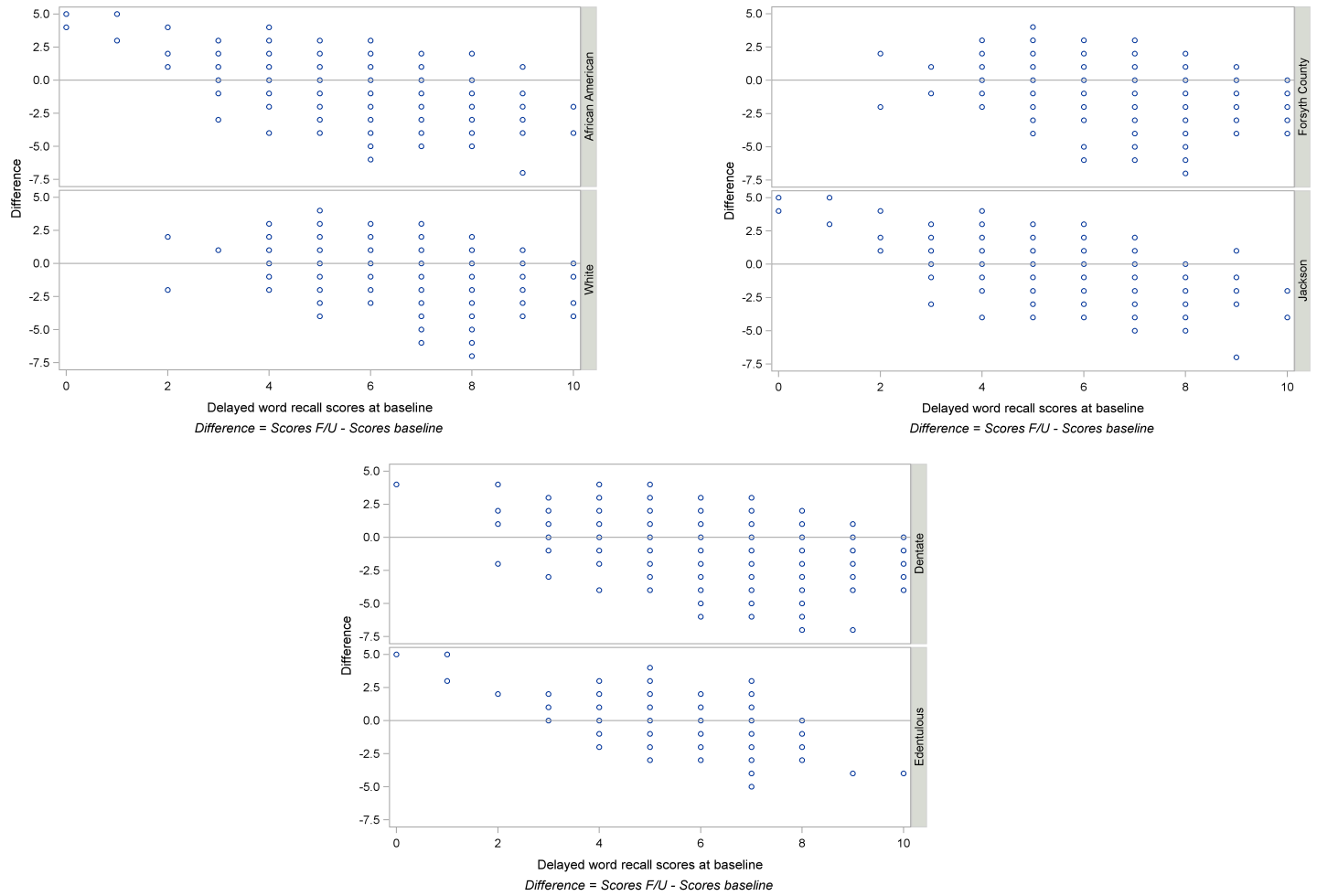


Figure 5-8**.** Scatterplots for the association between baseline cognitive scores and change in the Delayed Word Recall by race, study sites, and dental status (n = 911)

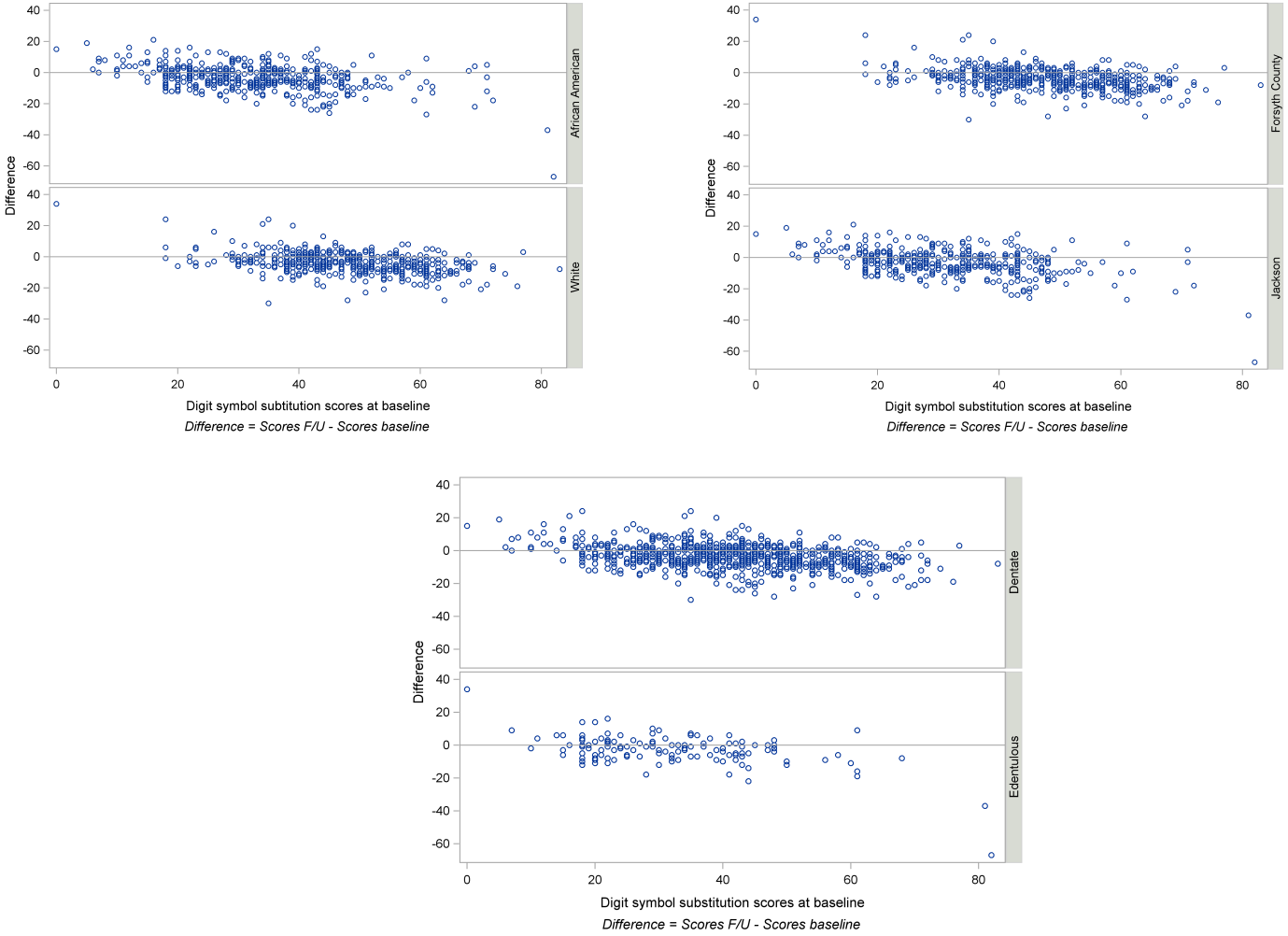


Figure 5-9. Scatterplots for the association between baseline cognitive scores and change in the Digit Symbol Substitution scores by race, study sites, and dental status dental status (n = 911)

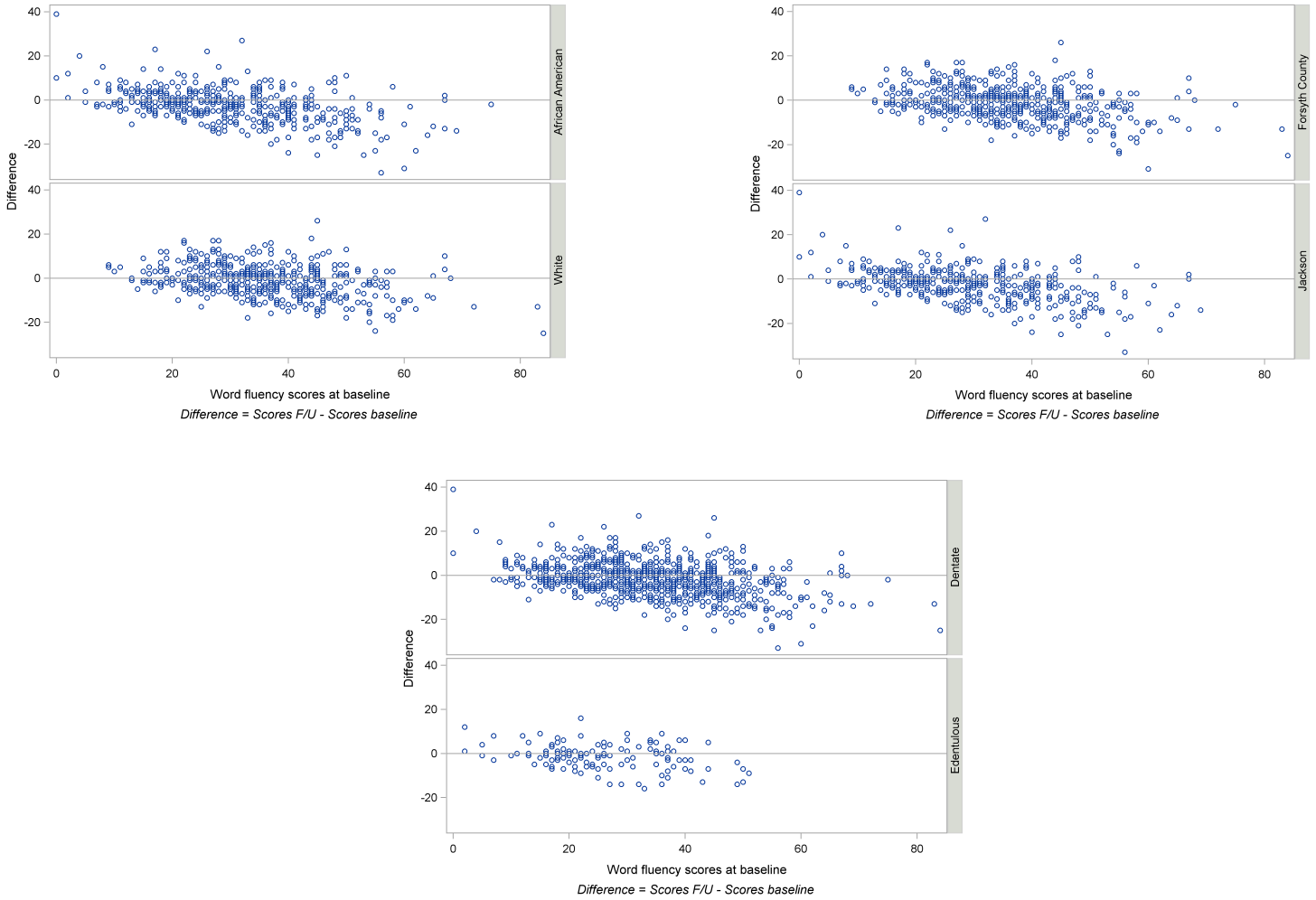


Figure 5-10. Scatterplots for the association between baseline cognitive scores and change in the Word Fluency scores by race, study sites, and dental status dental status (n = 911)

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DISCUSSION AND CONCLUSIONS

A. Summary of major findings

The present study was based on a conceptual framework that periodontal infection and inflammation in midlife are potential biological pathways for inducing or accelerating cognitive decline. Periodontal infection was assessed through a clinical periodontal examination, number of missing teeth, and also edentulism, since loss of teeth is an outcome of severe periodontal disease.

In this subset of the Atherosclerosis Risk in Communities (ARIC) cohort, baseline distributions for all three cognitive test scores were approximately normal (Gaussian). In general, cognitive ability was more strongly correlated with socio-demographic factors than medical health conditions. White participants tended to have better cognitive performance on all tests than African Americans, corresponding to the marked disparities in educational attainment and income.

In our cross-sectional study, complete tooth loss was consistently associated with lower performance on three measures of cognitive function whereas few teeth and periodontal disease were correlated only with low DSS and WF scores. The net differences in adjusted mean scores between dentate and edentulous participants were 0.16, 2.18, and 1.87 for the DWR, DSS, and WF tests, respectively. Although there was no clear dose-response relationship among the five levels of periodontal conditions, cognitive scores of participants with a greater extent of bleeding on probing (gingivitis and periodontitis with severe bleeding) were lower, on average, than scores of subjects who had less bleeding on probing, regardless of pocket depth.

These findings suggest that cognitive function was correlated with the host inflammatory response. Although the duration of having periodontal disease was not taken into account, it has been shown that the BGI index is correlated with a cumulative burden of periodontal disease and, indeed, the severe periodontitis group exhibited a greater extent of attachment loss (1). According to my conceptual model, the severe periodontitis group should have the lowest cognitive scores, given that participants in this subgroup had both chronic destructive periodontal disease and an active inflammatory state. However, the shallow lesions with bleeding that are classified as gingivitis may reflect deep lesions that have been treated and so now remain stable with gingival recession but with active inflammation.

To investigate the relation of cognitive scores to different components of the BGI, we conducted additional analyses using bleeding on probing, pocket depth, attachment loss, plaque score, and the CDC/AAP classification, which is based on a combination of probing pocket depth and attachment loss levels and has been used in NHANES. Our results (Supplemental Table 4-8) confirmed that poor performance in executive function tests was strongly correlated with the extent of bleeding on probing rather than with pocket depth, which means that the observed associations were mostly driven by BOP. In addition, poor performance in both memory and executive function tests was associated with attachment loss. Given the differences of underlying bacterial components and levels of periodontal tissue destruction between people who had periodontitis (severe, moderate, or low bleeding) and those who did not (gingivitis or healthy), in this case, we would see cognitive scores of gingivitis < healthy and severe < moderate < low bleeding among participants with periodontitis. But, it was not necessary that people with periodontitis/ severe bleeding scored lower than those with gingivitis or people with periodontitis/ low bleeding group scored lower than gingivitis group.

We also found that plaque deposits were negatively correlated with all cognitive function tests. The association between active periodontal disease and reduced cognitive function is consistent

with the concept that periodontal bacteria are involved in the pathophysiology of cognitive function changes and also the concept that low cognitive ability or cognitive decline taking place before oral health status assessments may lead to poorer oral hygiene, thereby increasing plaque deposits and gingival bleeding in shallow lesions or, perhaps, early gingivitis.

In our longitudinal study, a subset of study participants (n = 911) from two study sites (Forsyth County NC and Jackson MS) were available for the analysis. Over the eight-year follow-up, mean scores from all three cognitive measures decreased slightly. Overall declines in cognitive scores were 0.7, 3.6, and 1.8 points for the DWR, DSS, and WF tests, respectively. Although we found that complete tooth loss was associated with low performance on two cognitive tests, the DWR and WF, our data did not support the hypothesis that poor oral health predicted greater cognitive decline. Moreover, edentulous participants seemed to exhibit less cognitive decline than did dentate subjects, especially for the DWR test. Number of teeth and periodontal disease were not associated with cognitive performance nor with a steeper age-related cognitive decline over the eight years of follow-up.

Although our cross-sectional and longitudinal findings appear inconsistent, the longitudinal study (drawn from the brain MRI subcohort) had a relatively small sample size, and the two study populations differed in socio-demographic characteristics, cognitive performance at baseline, and oral health status (Table 7-12). For example, participants in the longitudinal study were more likely to be African American (48.5%), female, and older, and had lower incomes but higher educational attainment. DWR and WF scores at baseline were similar for the two studies, but the longitudinal study subjects had DSS scores that were approximately three points lower than subjects in the cross-sectional study. The prevalence of complete tooth loss (13.4 % in cross-sectional *vs.* 13.8% in cohort) and severe periodontal disease (periodontitis with severe bleeding, 12.3 % *vs.* 13.3 %) did not differ. However, the prevalence of gingivitis (gingivitis) in the longitudinal study (28.3 %) was approximately twice that in the cross-sectional study (14.9 %). We also acknowledge that the

longitudinal study, with only 911 participants, may have been underpowered to detect weak associations of number of teeth and periodontal disease with cognitive function.

B. Strengths

Although the longitudinal study population was small, the cross-sectional study analyzed a large population-based sample that included predominantly late middle-aged adults receiving a comprehensive evaluation of periodontal disease status. Prior studies reporting associations between oral health and cognitive function have almost exclusively investigated older populations, where severe cognitive decline may already be present (2-5). With older participants poor oral health is more likely to reflect functional disability, poor oral self-care, and less frequent use of dental services (6,7). The fact that we found cross-sectional associations between oral health (i.e., complete tooth loss) and cognitive function in midlife makes it less likely that these associations can be attributed primarily to adverse effects of severe cognitive dysfunction on oral health. If the mechanism behind the association begins with cognitive impairment, an extended interval would be needed for the subsequent decline in oral health to translate into tooth loss and edentulism. In addition, almost half of our edentulous participants reported initial diagnosis of gum disease in mid-thirties.

To our knowledge, our study is the first to examine the association between periodontal disease, as classified by the BGI index, and cognitive function. A primary advantage of using the BGI index is that we are able to study the association between natural gradients of periodontal disease severity and cognitive function. The five levels of the BGI differ in their underlying biological processes, which include the microbial, inflammatory, and acquired immune response. For example, BGI-G, BGI-DL/MB, and BGI-DL/SB subjects were more likely to have increased titers of *C. rectus* IgG. The two latter groups were more likely to show elevated titers of *P. gingivali* IgG. Importantly, the BGI index was developed using biospecimens from and clinical evaluation conducted on Dental

ARIC participants (1). Thus, the applicability of the BGI definition to our study sample is not an issue.

Other strengths of the present study include high quality control for periodontal and cognitive data. All comprehensive dental examinations and cognitive function tests were carried out by well-trained examiners using standard protocols. A high percentage of agreement between examiners was achieved (8,9). Periodontal probing was performed at six sites per tooth for all remaining teeth. Therefore, misclassification of periodontal disease condition is unlikely. In addition, since Dental ARIC provided extensive data on the clinical signs of periodontal disease, we were able to explore a variety of clinical definitions that may be predictive of cognitive function and change.

In our study, directed acyclic graphs (DAGs) and the change-in-estimate procedures were used for adjustment-variable selection. DAGs were created based on assumptions about the exposure-outcome relationship to identify variables to adjust for confounding and other biases. Nonetheless, there is uncertainty that the *a priori* DAG fits with the confounding pattern of the dataset. For instance, body mass index in the ARIC data was a significant confounder for oral health measures and cognitive outcome associations in the bivariate analyses, but was not included in the minimally sufficient adjustment variables set. In this case, model selection based on the change-in-estimate, which allows patterns in the data to decide the final adjustment variables, may include BMI in the final adjustment set. However, this approach has drawbacks if variables are measured with errors and variables associated only with the outcome alone may be selected. The combination of these two approaches leads to a more parsimonious model when compared to a selection based on the change-in-estimate only since the model chooses variables by relevance to the exposure-outcome relationship. However, the change-in-estimate procedures allows us to check uncertainties underlying the *a priori* assumptions.

C. Limitations

Four limitations of the present study deserve consideration. The primary limitation of our study is generalizability. Although we used data from a large population-based study, the generalizability of our research findings to the U.S. population is limited since study participants were sampled from only four geographic areas in the U.S. (i.e., Forsyth County, NC; Jackson, MS; the Northwest suburbs of Minneapolis, MN; and Washington County, MD) and the analytical samples were confined to only white and African American participants. Furthermore, only ARIC participants from the Forsyth County and Jackson study centers were available for our prospective study.

Second, measures of cognitive function included three tests, which assessed only two cognitive domains, memory (DWR) and executive function (DSS and WF). Our prospective study was based on cognitive function assessments at two time points. It has been acknowledged that the most effective way to characterize real cognitive decline is by analyzing change over several assessments. Assessments at two time points provide limited ability to differentiate true change from changes due to random variation, such as learning effects, random fluctuations, and measurement error. In ARIC, the correlation coefficient between two measurements with an average interval of 490.75 days for DWR test ($R^2 = 0.55$) was moderate, whereas correlation coefficients of DSS ($R^2 = 0.81$) and WF tests ($R^2 = 0.84$) were high (ARIC quality control report; unpublished data). Scatterplots displaying the difference in cognitive scores (scores at follow-up and scores at baseline) suggested that participants with higher cognitive scores at baseline tended to have greater cognitive declines.

Third, the dental examination was restricted to participants who did not require antibiotics before dental procedures. This exclusion could lead to an underestimation of the association between periodontal disease and cognitive decline if people who require antibiotic prophylaxis have medical conditions that are associated with severe periodontal disease. Oral health measures in the data set

were available only at Visit 4. Therefore, we cannot assess the effects of cognitive function on tooth loss or periodontal disease status that may have occurred later in life. It is therefore possible that deterioration of oral health might be a result of impaired cognition. Furthermore, we were unable to take into account the duration of having periodontal disease or previous periodontal treatment. It is likely that subjects who periodically visit dentists will exhibit a lower level of periodontal inflammation, even if they have pocket lesions.

Lastly, it has been acknowledged that poor oral health can be a result of severe adverse conditions, including compromised systemic health and low socio-demographic status, which are known risk factors for cognitive deficits. Although we carefully included all measured confounders and modifiers in the analyses, biased estimates from unmeasured confounders are still possible. In addition, we did not investigate whether tooth loss was also associated with dietary changes or nutritional status, which may play a role in the onset of cognitive decline.

D. Public health significance

Oral health is recognized to be an important determinant of general health and quality of life. Periodontal disease is a common oral disease and an important source of chronic infection and inflammation in adults. The estimated prevalence of periodontal disease in the U.S. ranges from 24.4% in adults 30-34 years old to 70.1% in adults aged 65 years and older (10). Tooth loss secondary to poor oral health, including severe periodontal disease, may lead to worsening nutritional status. Both systemic infection and inflammation as well as nutritional deficiency have been implicated as possible causal pathways for cognitive decline and dementia. At present, these two chronic conditions are major public health concerns affecting older adults in the U.S. and worldwide because there is no currently effective treatment or prevention for cognitive impairment and dementia. The prevalence of all types of dementia ranges from approximately 2% in people aged 65-69 up to more than 30% in people over 90 years of age (11). In order to prevent or delay the clinical onset of cognitive

impairment and dementia, efforts to identify modifiable factors are, therefore, essential. The relationship between periodontal disease and cognitive function is potentially a critically public health problem due to the high prevalence of both conditions. If poor oral health in midlife has an important role in the pathophysiology of cognitive impairment and dementia, periodontal treatment or oral hygiene improvement would be a promising avenue to help reduce the burden of these chronic conditions.

E. Future research direction

Further investigation into a possible link of periodontal disease and/or tooth loss with cognitive function is warranted. Most current findings, including those from our studies, suggest that tooth loss is associated with cognitive function independent of known confounders. However, the nature of the association between periodontal status and cognitive function remains unclear. We have shown that the inflammatory state of periodontal disease is related to cognitive status, supporting the inflammatory model hypothesis. However, our cross-sectional study cannot demonstrate that periodontal disease is a cause of cognitive decline. Other potential mechanisms must still be considered.

In fact, periodontal disease is a time-varying disease. To examine the temporal relationship and evaluate whether periodontal disease is independently associated with cognitive decline and dementia, a large population-based, prospective study that enrolls participants in midlife and obtains repeated measurements of cognitive function and periodontal status would be an appropriate design. Uniform criteria for periodontal disease case definition and disease severity would improve future studies. Since periodontal disease involves a complex interaction of biofilm with both the host inflammatory and immune responses, individuals vary in susceptibility to periodontal disease due to oral hygiene status and genetic backgrounds. It is, therefore, necessary to consider different clinical manifestations of periodontal disease together with systemic inflammatory markers and genetic

factors in response to periodontal infection. To our knowledge, there is only one pilot study that has examined an effect of oral care, specifically tooth brushing, on preventing subsequent cognitive degradation in demented patients (12), and none has investigated an effect of periodontal treatment. Further investigation in well-conducted controlled intervention studies is necessary to prove a benefit of periodontal intervention, i.e., that periodontal disease treatment and long-term maintenance have changed the levels of systemic inflammation and reduced the risk of cognitive impairment and dementia in later life.

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Table A-1. Cardiovascular and other non-oral health risk factors for cognitive decline, dementia, and brain abnormalities identified in the ARIC studies

Studies	Study design	N	Major risk factors	Outcome	Results
Knopman *et. al.,* 2011 (1)	Longitudinal Visit 3 (1993-1994) to 2004-2006	1,112	Diabetes, hypertension, fasting blood sugars, systolic blood pressure, stroke, APOE genotype	Infraction WMHs, VS, SW,	Diabetes was associated with incident infarcts (OR 1.95, 95% 1.29-2.95) and worsening SW (OR 2.0, 95% 1.36-3.24). Hypertension was associated with incident infarcts (OR 1.73, 95% CI 1.23-2.42). APOE ε4 allele was not associated with brain abnormalities.
Christman *et. al*., 2011 (2)	Longitudinal Visit 2 (1990-1992) to Visit 4(1996-1998)	8,958	Diabetes, Hyperglycemia;HbA_{1c}	DWR, DSS, WF, Dementia hospitalization	Diagnosed diabetes was associated with cognitive decline on the DSS test (OR 1.42, 95% CI 1.14-1.75) and dementia incidence (HR 2.82, 95% CI 1.83-4.30). HbA_{1c} was a significant predictor for cognitive decline among people with diabetes.
Knopman *et. al*., 2009 (3)	Longitudinal Visit 2(1990-1992) to 2004-2006	1,130	Diabetes, hypertension, stroke, Plasma lipids, Lipoproteins, APOE genotype, metabolic syndrome[2]	DWR, DSS, WF	Diabetes and APOE genotype were associated with a decline in performance on the DSS test, whereas hypertension and stroke were not. For DWR, stroke and APOE genotype predicted cognitive decline. For the WF, metabolic syndrome, hypertension, and stroke were associated with decline.
Alonso *et. al*., 2009 (4)	Longitudinal Visit 2(1990-1992) to 2004	11,151	Smoking, hypertension, diabetes, BMI, hypercholesterolemia, APOE genotype	Dementia	There were 203 cases of hospitalization with dementia. Smoking (HR 1.7, 95% CI 1.2-2.5), hypertension (HR 1.6, 95% CI 1.2-2.2), diabetes (HR 2.2, 95% 1.6-3.0), and APOE genotype (HR 1.8, 95% CI 1.4- 2.4) were risk factors for dementia. The associations of BMI and hypercholesterolemia with dementia were not significant.
Young *et.al*, 2006 (5)	Longitudinal Visit 2(1990-1992) to Visit 4(1996-1998)	7,148	Hyperinsulinemia	DWR, DSS, WF (DWR < 3, dementia)	Hyperinsulinemia was associated with lower DWR, DSS, and WF scores at baseline and a greater decline over 6 years in DWR and WF.
Blair *et.al.,* 2005 (6)	Longitudinal Visit 2(1990-1992) to Visit 4(1996-1998)	7,895	APOE genotype, diabetes, hypercholesterolemia	DWR, DSS, WF	The association between APOE genotype groups with all cognitive function tests was observed among Caucasian individuals, while the result was inconsistent among African-

					American individuals. People with APOE ε4 had greater cognitive decline for DWR and DSS. The combination APOE ε4 with hypercholesterolemia or diabetes showed the greatest cognitive decline.
Knopman *et.al.,* 2001 (7)	Longitudinal Visit 2(1990-1992) to Visit 4(1996-1998)	10,963	Hypertension, diabetes, hyperlipidemia, smoking, carotid artery IMT, NSAID	DWR, DSS, WF	Decline in cognitive function was greater in participants with hypertension, diabetes, or incident stroke. The strong associations were observed between cardiovascular risk factors and DSS test.
Knopman *et.al.,* 2005 (8)	Cross-sectional Visit 3(1993-1995)	1,812	Diabetes, fasting blood sugar, BMI, APOE genotype, hypertension, blood pressure, smoking, alcohol intake	VS, SW	Diabetes and increased fasting blood sugar were associated with high grade VS (OR 1.07, 95% CI 1.19-2.24 and OR 1.07, 95% CI 1.19-2.24, respectively)
Mosley *et.al.,* 2005 (9)	Cross-sectional Visit 3(1993-1995)	1,528	WMHs, SW, VS	DWR, DSS, WF	The presence of two or more high-grade abnormalities was associated with increased risks of impaired functioning on the DWR (OR 2.23, 95% CI 1.40-3.55); DSS (OR 2.06, 95% CI 1.13-3.76); and WF (OR 2.07, 95% CI 1.23-3.49).
Ding *et.al*., 2003 (10)	Cross-sectional Visit 3(1993-1995)	1,909	Alcohol intake	WMHs, VS, SW infraction	There was no association between alcohol intake and the presence of MRI infraction. Alcohol intake was not associated with WMHs grades, but with higher grades of VS and SW.
Cerhan *et.al*., 1998 (11)	Cross-sectional Visit 2(1990-1992)	13,913	Depressive symptoms, current smoker, sport index, pulmonary function, alcohol drink, pulmonary function, fibrinogen, carotid artery IMT, diabetes, hypertension	DWR, DSS, WF	The DWR was negatively associated with depressive symptoms, diabetes, and fibrinogen. The DSS was negatively associated with depressive symptom, smoking, fibrinogen, and carotid IMT, and positively associated with alcohol drinking and pulmonary function. The WF was positively associated with alcohol drinking and sports participation.

DWR, Delayed word recall; DSS, Digit symbol substitution; HbA1c, Glycated haemoglobin; IMT, Carotid artery intima-media thickness; NSAID, Non-steroidal anti-inflammatory drug; WF, Word fluency; WMHs, White matter hyperintensities; VS, Ventricular size; SW, Sulcal width.

[1]Study participants aged 45-64 at inception (1987-1989; Visit 1).

[2]Metabolic syndrome was defined as any three of the following: fasting blood sugar > 110 mg/dL or the use of an antidiabetic agent; triglycerides > 150 mg/d; high-density lipoprotein (HDL) cholesterol level < 40 mg/dL in men or < 50 mg/dL in women; hypertension (systolic blood pressure > 130 mm Hg or diastolic blood pressure >85 mm Hg or current use of an antihypertensive agent); or waist circumference > 88 cm in women, or > 102 cm in men.

Table A-2. Cognitive decline and dementia are associated with an increased likelihood of poor oral health

Authors	Study design	N	Age	Cognitive assessment	Oral health measures	Results
Syrjala *et.al*., 2012 (12)	Cross-sectional	354	≥75	Dementia types	Caries, PPD > 4 mm, edentulousness, oral hygiene, denture hygiene	Participants with AD and other dementia had an increased risk of poor oral health (more dental caries, less teeth, more teeth with deep PPD) and oral hygiene.
Ellefsen *et.al*., 2009 (13)	Longitudinal (1-yr F/U)	106	81.9	MMSE Dementia IADL	Caries increment	Compared with non-demented group, individuals with AD and other dementia had higher adjusted caries increments (3.3 surfaces for non-demented; 4.7 and 5.8 surfaces for AD and other dementia, respectively).
Chalmers *et.al*., 2005 (14)	Longitudinal (1-yr F/U)	224	≥75	MMSE ADL	Caries incidence Caries increment	The coronal (64.4%) and root caries (48.5%) incidence were high. The adjusted coronal and root caries increments were 2.5 and 1.0 surfaces, respectively.
Chalmers *et.al*., 2003 (15)	Longitudinal (1-yr F/U)	132	≤ 79 ≥ 80	MMSE IADL, ADL	Coronal and root caries attack rate[1], oral mucosal lesions, retained root, tooth loss, use of dentures, plaque index	Participants with dementia had poorer oral conditions than those without dementia. Coronal and root surface caries as well as plaque score were higher in participants with moderate-severe dementia and functionally impairment.
Yu *et.al*., 2008 (16)	Cross-sectional (NHANE 2001-02)	803	≥60	DSS[2] (Individual standard deviation score)	PD[3]	Higher cognitive function were associate with lower odd of PD (OR 0.69, 95% CI 0.51-0.94).
Adam *et.al*., 2006 (17)	Cross-sectional	135	≥65	AMT	Caries, tooth loss, plaque deposit, denture	The number of caries and missing teeth as well as mean dental plaque and calculus were similar for both no/mild and moderate/ sever dementia. Mean number of missing teeth was 28.22 and 27.88 for no/mild and moderate/severe dementia, respectively.

AD, Alzheimer's disease; ADL, Activities of daily living scale; AMT, Abbreviated mental test; DDST, Digit symbol substitution; IADL, Instrumental activities of daily living; MMSE, Mini-mental state examination; PD, Periodontal disease; PPD, periodontal pocket depth

[1]Coronal and root caries attack rate were the number of tooth surfaces affected by caries as a percentage of the total number of surface at risk.

[2]Participants were asked to copy symbols that were paired with numbers within 2 minutes.

[3]Periodontal disease was defined as at least 10% of sites with clinical attachment loss of more than 4 mm and at least 10% sites with probing depth greater than 3 mm.

Table A-3. Possible causal relationships of periodontal disease or tooth loss with cognitive decline

Authors	Study design	N	Age	Markers/ Genotypes	Oral health measures	Outcomes	Results
Arrive *et.al.,* 2012 (18)	Longitudinal (15-yr F/U)	405	66-80		DMFT, POP, CPI	Alzheimer Dementia	Seventy-two persons developed a dementia during a median follow-up of 10 years. Among people with low school level, missing teeth ≥11 was associated with the lower risk of dementia (HR 0.40, 95% CI 0.17-0.94). The periodontal condition and masticatory function measured by POP were not associated with the risk of dementia.
Batty *et.al.,* 2011 (19)	Longitudinal in type 2 diabetes patients (5-yr F/U)	11,140	55-88		Self-reported number of teeth, number of days of bleeding gums	MMSE Dementia	Having no teeth was associated with the highest risk of both dementia (HR 1.48, 95% CI 1.24-1.78) and cognitive decline (HR 1.39, 95% CI 1.21-1.59). Number of days of bleeding gums was unrelated to the outcomes.
Kaye *et.al.,* 2010 (20)	Longitudinal (32-yr F/U)	597	28-70		Rates of tooth loss, caries incidence, probing depth, alveolar bone loss	MMSE SCT	Higher rates of tooth loss and periodontal disease progression during adulthood predicted performance on the MMSE and SCT.
Sein *et.al.,* 2010 (21)	Longitudinal "The Nun study"	144	75-98	APOE	Number of teeth	DWR	Individuals with both APOE and fewer teeth had lower DWR at baseline and declined more quickly compared with participants with neither of these risk factors or with either risk factor alone.
Starr *et.al.,* 2008 (22)	Longitudinal "The Healthy Old People in Edinburgh" (9-yr F/U)	201	≥70		Complete tooth loss	MMSE, NART, RPM, LM	Being edentulous was associated with lower MMSE ($p = 0.006$), RPM ($p = 0.028$), and LM ($p = 0.015$). These associations all became non-significant after adjusting for NART and age.

Stein *et.al.,* 2007 (23)	Longitudinal "The Nun Study"	144	75-98	APOE	Number of teeth, alveolar bone loss	MMSE, DWR, others[1]	A low number of teeth increased a prevalence (OR 4.30, 95% CI 1.16-15.60) and an incidence of dementia (HR 2.2, 95% 1.10, 4.50).
Shimazaki *et.al.,* 2001 (24)	Longitudinal (6-yr F/U)	1,929	≥ 65		Number of teeth	Mental health status[2]	The OR for mental impairment of edentulous subjects not using dentures to the subjects with 20 or more teeth was 2.4 (95% CI 0.9-6.5).
Matthews *et.al*., 2011 (25)	Cross-sectional	9,583	45- ≥85	Serum CRP	Self-reported tooth loss	WLL	Higher number of tooth loss (> 16 teeth) was associated with poorer cognitive function. However, the association was not significant after adjusting for demographic factors, socio-economic status, and other risk factors (i.e. BMI, CRP).
Kamer *et.al.,* 2012 (26)	Cross-sectional	152	70		MCPI, number of missing teeth	WAIS[3]	Periodontal inflammation (MCPI ≥ 3) and high number of missing teeth (≥ 11) were associated with lower cognitive scores. Education and cognitive scores at age 50 were important confounders.
Rai *et.al.,* 2012 (27)	Cross-sectional	55:PD 20:Dementia 32:Controls	60-69 59-69 58-69	WBCs, neutrophils, thrombocytes, CRP, MMP-8, MMP-9, IGF-I, TNF-α, GCF MMP-8 and MMP-9	Plaque, GI, PPD, CAL, horizontal bone loss, number of teeth	Dementia	Dementia and periodontitis patients had poorer oral health than healthy controls. Total WBCs, neutrophil, thrombocytes, CRP, serum and GCF MMP-8 and MMP-9 levels were higher in dementia and periodontitis patients compared to controls.
Okamoto *et.al*., 2010 (28)	Cross-sectional	4,206	≥ 65		Number of teeth, length of edentulous period, CPI[4]	MMSE GDS	ORs of 0-10 vs. 22-32 remaining teeth were 1.68 (95% CI 1.07-2.63) for mild memory impairment, and 2.17 (95% CI 1.51-3.14) for a low MMSE. ORs of ≥ 15 vs. < 15 years edentulous period for low MMSE was 3.10 (95% CI 1.43-6.7).
Grabe *et.al.,* 2009 (29)	Cross-sectional	1,336	60-79		Number of teeth	MMSE	A decreased number of teeth was associated with lower MMSE scores in female (p = 0.002) but not in males (p = 0.825).

Noble *et.al*., 2009 (30)	Cross-sectional: NHANES III	2,355	≥60	*P. gingivalis* Ig G		Logical verbal memory test[5], serial subtraction	Individuals with the highest *P. gingivalis* IgG had significantly greater odds of poor DWR (OR 3.01, 95%CI 1.06-8.53) and impaired subtraction (OR 2.00, 95% CI 1.19-3.36).
Stewart *et.al.,* 2008 (31)	Cross-sectional: NHANE III	5,138 1,555	20-59 ≥70		Gingival bleeding, CAL ≥ 3 mm, number of missing teeth	SDST, SDLT, A story recall test	Gingival bleeding and CAL were associated with SDST (ß = 0.003), and gingival bleeding was associated with SDLT (ß = 0.017).
Stewart *et.al*., 2007 (32)	Cross-sectional	4,032	≥65		Complete tooth loss	AMTS	Lack of teeth was associated with cognitive impairment (OR 3.59, 95% CI 2.36-5.47).
Kim *et.al*., 2007 (33)	Cross-sectional	686	≥65		Number of teeth	MMSE Dementia diagnosis	The prevalence of dementia increased for each decreasing quintile of tooth loss (OR 1.4, 95% CI 1.1-1.7).
Gatz *et.al.,* 2006 (34)	Case-control	310 cases 3,063 controls (106 identical twins)	≥65		Tooth loss before age 35	Dementia diagnosis	History of tooth loss before age 35 and low educational attainment were significant risk factors for AD.

AMTS, The abbreviated mental test score; CAL, Clinical attachment level; CPI, Community periodontal index; CRP, Serum C-reactive protein; DMFT, Number of decayed (D), missing (M), or filled (F) teeth (T) index; GI, Gingival index; PPD, probing pocket depth; DWR, Delayed word recall; GCF, Gingival crevicular fluid; GDS, Geriatric depression scale; IGF, Insulin-like growth factor; LM, Logical memory test; MCPI, Modified community periodontal index; MMP, Matrix metalloproteinase; MMSE, Mini-mental state examination; NART, the national adult reading test; POP, Number of posterior occluding pair; RPM, Ravens progressive matrices; SCT, Spatial copying task; SDLT, The serial digit leaning test; SDST, The symbol digit substitution test; TNF, Tumor necrotic factor; WBCs, Total count of white blood cells; WLL, Word list learning.

[1]The Boston naming test, the verbal fluency test, and constructional praxis test.

[2]Mental health status was classified into three categories according to subject's symptoms of dementia and degree of cognition: good, fair, and poor.

[3]Four subsets of the Wechsler Adult Intelligence Scale (WAIS) were measured: digit span, digit symbol (DST), picture completion, and block design (BDT).

[4]Community periodontal index (CPI); 0 = healthy, 1 = gingival bleeding after probing; 2 = calculus present in the periodontal pocket; 3 = periodontal pocket 4-5 mm; 4 = periodontal pocket at least 6 mm.

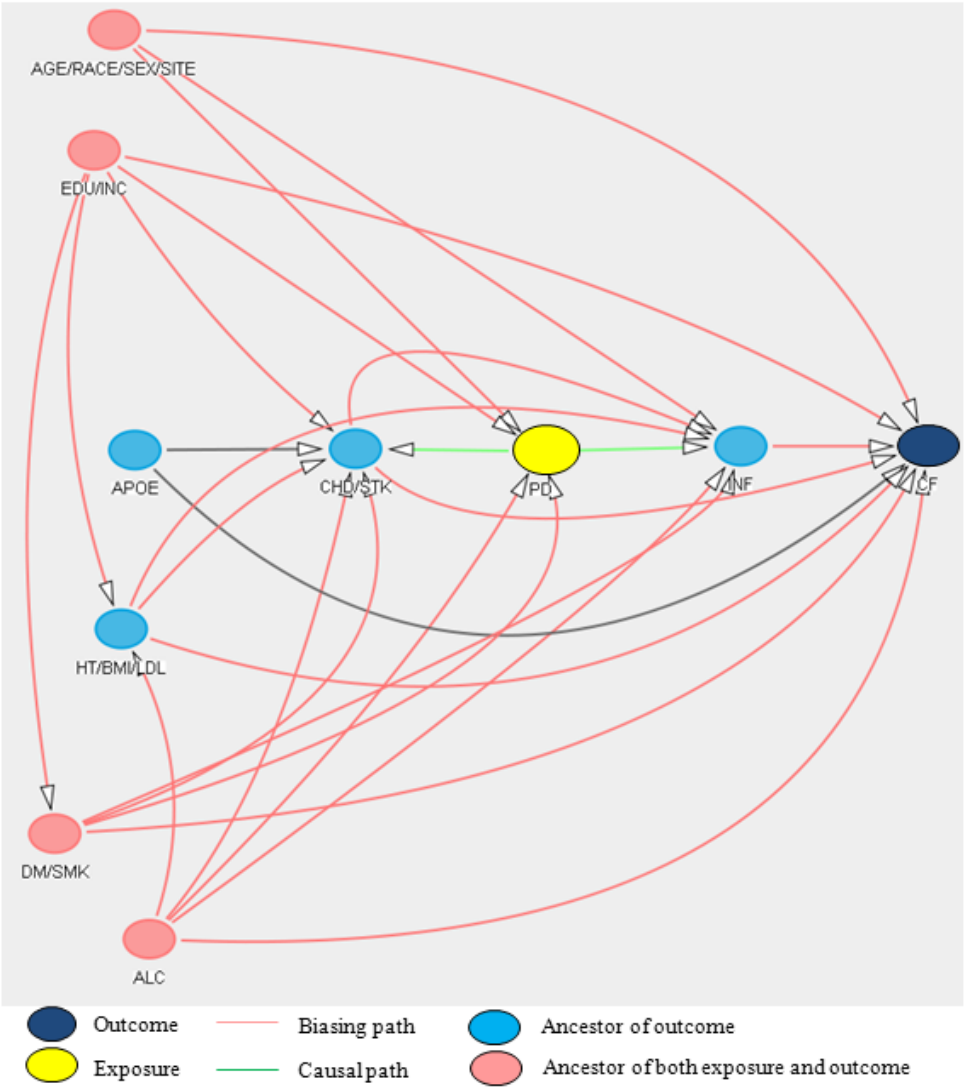
[5]An immediate and delayed logical verbal memory test from the East Boston memory test.

Table A-4. Clinical measures, inflammatory biomarkers, and microbiology components of periodontal diseases: Dental ARIC

Author	N	Exposure	Outcome	Covariates	Results
Zhong *et.al.,* 2007 (35)	6,277	Maximum PPD Maximum CAL BOP	GCF IL-1β, PGE_2	Age, gender, race, BMI, dental visit, tooth brushing, NSAID, BOP, smoking, diabetes,	GCF IL-1β, PGE_2 were more strongly associated with maximum PPD and BOP than CAL.
Offenbacher *et.al.,* 2007 (36)	6,768	GCF IL-1β, PGE_2, IgG levels to *P. gingivalis, C. rectus*	BGI index (BOP, PPD)	Age, gender, race, education, smoking, diabetes, BMI, brushing, flossing, dental visit	BGI-Deep lesion/ severe bleeding had an excessive IL-1β and PGE_2, and increased levels of IgG to *P. gingivalis* and *C. rectus.*
Beck *et.al.,* 2005 (37)	4,846	IgG to 17 oral microorganisms CDC/AAP classification	CHD	Age, gender, diabetes, hypertension, waist-to-hip ratio, HDL, LDL, education, smoking	Clinical signs of PD were not associated with CHD, while systemic antibody response was associated with CHD.
Kshirsagar *et.al,* 2005 (38)	5,537	CDC/AAP classification	GFR Serum creatinine	Age, gender, race, diabetes, hypertension, BMI, education, smoking, and serum CRP	Initial and severe PD were associated with higher odds of GFR less than 60 ml/min/1.73 m^2 after adjusting for all covariates (OR 2.00; 95% CI 1.23-3.24 for initial PD; OR 2.14; 95% CI 1.19-3.85 for severe PD).
Slade *et.al*., 2003 (39)	5,552	PPD ≥ 4mm (0-30% and > 30%)	Serum CRP	Age, gender, diabetes, smoking, NSAID, BMI	The association between PPD and serum CRP was modified by BMI level; when BMI was 35, the difference of serum CRP level between two groups of PPD was negligible.
Beck *et.al*., 2002 (40)	5,400	PPD BOP CAL	Serum CRP, ICAM1	Age, gender, race, triglyceride, diabetes, BMI, number of teeth, cholesterol medications, smoking, study sites, income, NSAID, arthritis, kidney infection, bronchitis, and sinus infection	PPD and BOP were strongly associated with systemic inflammation.

BGI, Biofilm-Gingival Interface; BOP, bleeding on probing; BMI, body mass index; CAL, clinical attachment level; CDC/AAP, The Centers for Disease Control and Prevention/ The American academy of Periodontology; CRP, C-reactive protein; CHD, Coronary heart disease; GCF, Gingival crevicular fluid; GFR, Glomerular filtration rate; HDL, High density lipoprotein; ICAM, Intracellular adhesion molecule; IgG, Immunoglobulin G; IL- Interleukin-1b ; LDL, Low density lipoprotein; NSAID, Non-steroidal anti-inflammatory drug; PD, Periodontal disease; PGE_2, Prostaglandin E_2; PPD, Probing pocket depth.

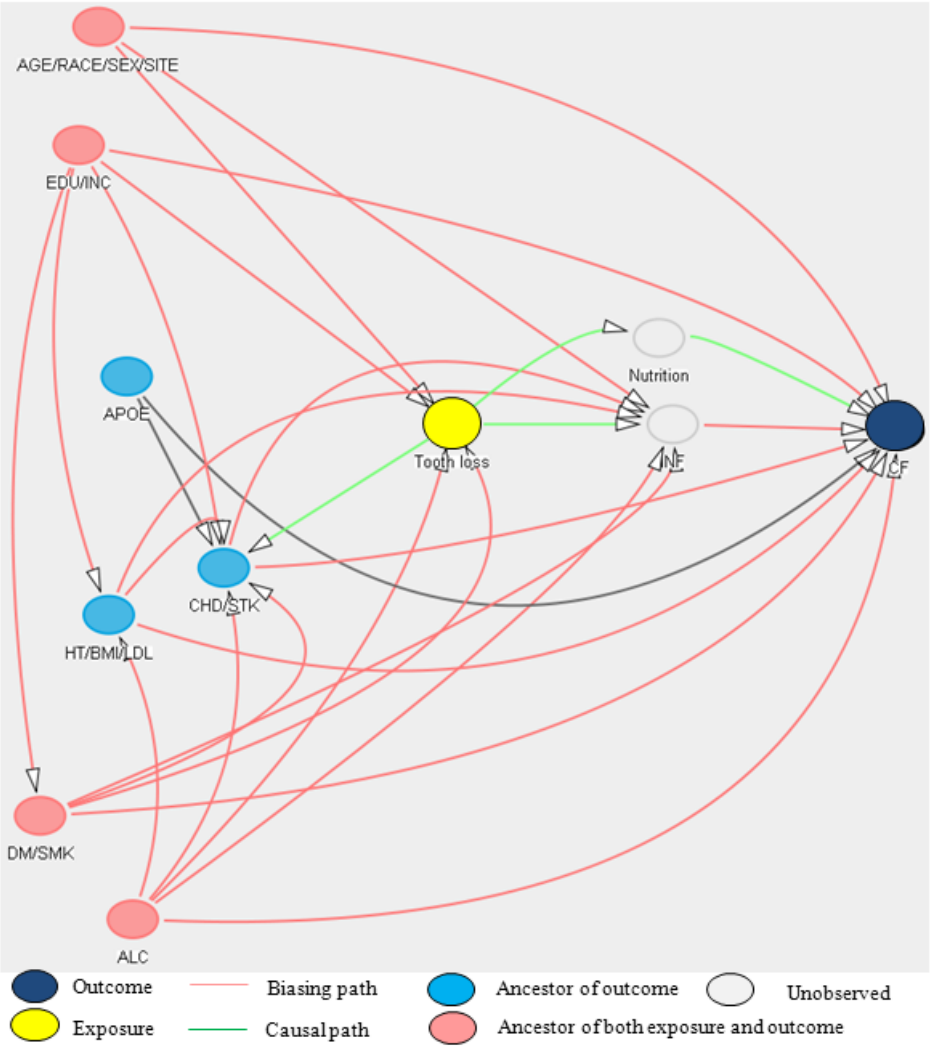
Appendix B: Directed acyclic graphs (DAGs)



A minimal sufficient adjustment set for estimating the effect of periodontal disease on cognitive function is {AGE, RACE, SEX, SITE, EDU, INC, DM, SMK, ALC}.

Figure B-1. Directed acyclic graph for associations between periodontal disease and cognitive function[1]

[1]EDU = education, INC = income, SITE = study site, APOE = apolipoprotein E, HT = hypertension, BMI = body mass index, LDL = low density lipoprotein, DM = diabetes mellitus, SMK = smoking, ALC = alcohol, CHD = coronary heart disease, STK = stroke, INF = inflammatory markers, CF = cognitive function, PD = periodontal disease



A minimal sufficient adjustment set for estimating the effect of tooth loss on cognitive function is {AGE, RACE, SEX, SITE, EDU, INC, DM, SMK, ALC}.

Figure B-2 Directed acyclic graph for associations between tooth loss and cognitive function[1]

[1]EDU = education, INC = income, SITE = study site, APOE = apolipoprotein E, HT = hypertension, BMI = body mass index, LDL = low density lipoprotein, DM = diabetes mellitus, SMK = smoking, ALC = alcohol, CHD = coronary heart disease, STK = stroke, INF = inflammatory markers, CF = cognitive function

Appendix C: Oral health measures

Dental status

Table C-1. Selected baseline characteristics of ARIC study samples in relation to dental status

Characteristics	Cross-sectional study n = 9,909[1]			Longitudinal study n = 911[2]		
	Prevalence (row %)		*P-value*[3]	Prevalence (row %)		*P-value*[3]
	Edentulous	Dentate		Edentulous	Dentate	
Age at Visit 4 (years)						
> 65	17.2	82.8	<0.0001	14.8	85.2	0.6030
60 - 65	12.4	87.6		12.7	87.3	
51 - 59	9.7	90.3		13.6	86.4	
Race						
African American	19.4	80.6	<0.0001	20.6	79.4	<0.0001
White	11.8	88.2		7.5	92.5	
Gender						
Male	13.0	87.0	0.3111	11.1	88.9	0.0563
Female	13.7	86.3		15.6	84.4	
Study sites						
Forsyth	11.9	88.1	<0.0001	8.7	91.3	<0.0001
Jackson	19.9	80.1		20.6	79.4	
Minneapolis	5.4	94.6		0	0	
Washington	19.0	81.0		0	0	
Education						
Less than high school	33.0	67.0	<0.0001	29.7	70.3	<0.0001
High school completion	12.7	87.3		14.2	85.8	
Post-secondary education	5.2	94.8		6.6	93.4	
Income						
Refused	12.8	87.2	<0.0001	13.6	86.4	<0.0001
<$25,000	25.0	75.0		24.8	75.2	
$25-<$50,000	11.5	88.5		9.8	90.2	
$50,000 or more	4.6	95.4		3.2	96.8	
Cigarette use						
Current	23.1	76.9	<0.0001	26.9	73.1	<0.0001
Former	13.2	86.8		11.9	88.1	
Never	10.2	89.8		11.9	88.1	
Alcohol use						
Current	8.0	92.0	<0.0001	7.7	92.3	0.0002
Former	18.4	81.6		18.4	81.6	
Never	19.4	80.6		16.0	84.0	
Diabetes mellitus						
Yes	22.4	77.6	<0.0001	21.0	79.0	0.0047
No	11.7	88.3		12.4	87.6	
Hypertension						
Yes	16.1	83.9	<0.0001	20.0	80.0	<0.0001
No	11.0	89.0		9.1	91.9	
Coronary heart disease						
Yes	21.0	79.0	<0.0001	14.3	85.7	0.9340
No	12.7	87.3		13.8	86.2	
Stroke						
Yes	20.7	79.3	0.0017	17.6	82.4	0.6469
No	13.2	86.8		13.8	86.2	
Hyperlipidemia						
Yes	14.8	85.2	0.0005	13.2	86.8	0.7102
No	12.4	87.6		14.1	85.9	

Body mass index (km/m^2)						
≥ 30	16.7	83.3	<0.0001	19.9	80.1	0.0003
< 30	11.6	88.4		11.1	88.9	
APOE ε4						
Yes	13.2	86.8	0.6907	17.6	82.4	0.0302
No	13.5	86.5		12.1	87.8	

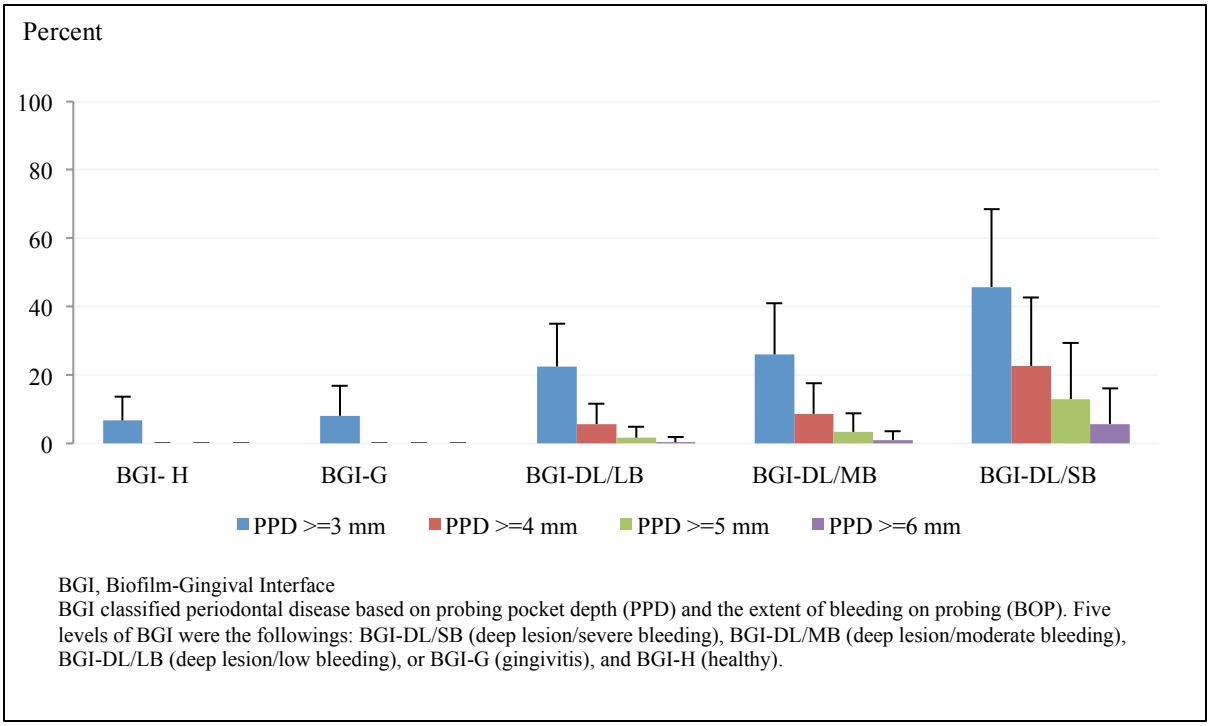
[1]Participants were from all four study sites. In the final analysis, African Americans from Washington and Minneapolis study sites (n =35) were excluded, leaving an analytical sample of 9,874.
[2]Paticipants were from two study sites (Forsyth County, NC and Jackson, MS).
[3]Chi-square test

Periodontal disease

Table C-2. Prevalence of periodontal diseases in Dental ARIC study, classified by CDC/AAP[1] index and Biofilm-Gingival Interface classification (n = 6,700)[2]

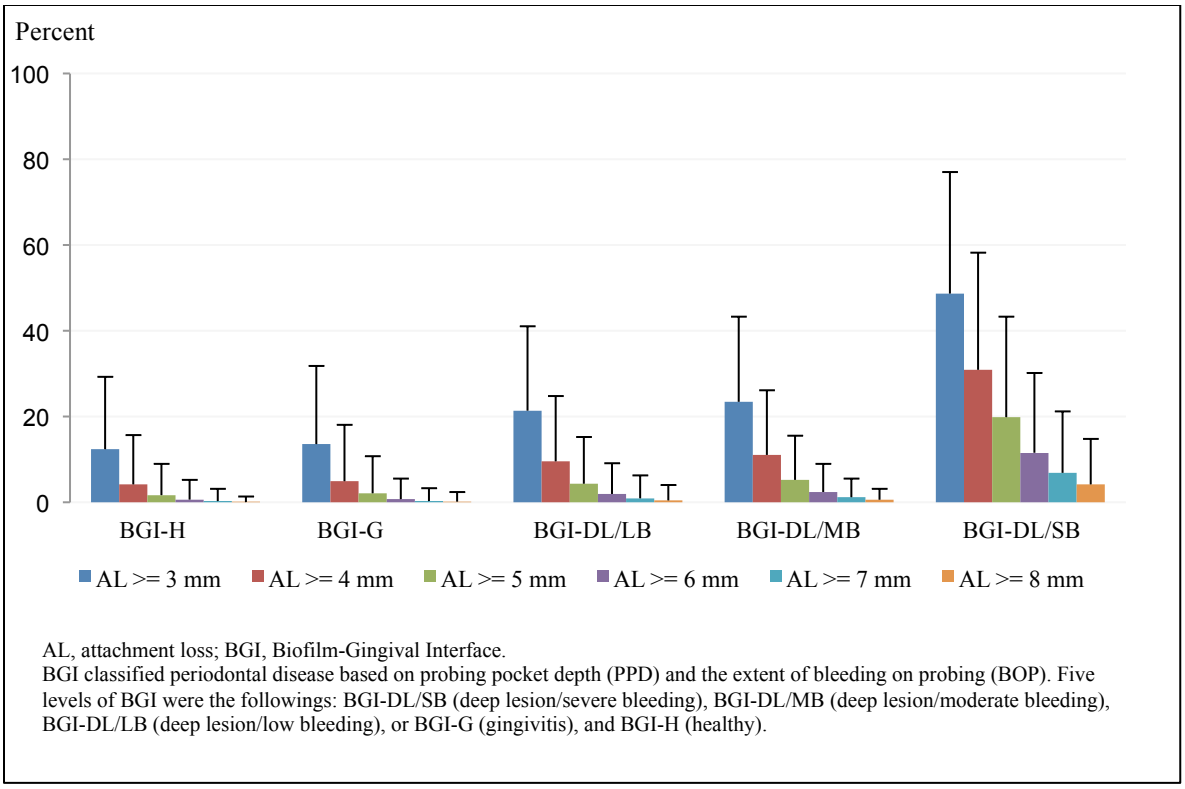
CDC/AAP classification	Biofilm-Gingival Interface (BGI) classification[3] (n, row %)										All	
	No periodontal pockets				Had periodontal pockets							
	BOP <10%		≥ 10%		BOP < 10%		10- < 50%		≥ 50%			
Healthy/Mild	786	28.4	823	29.7	405	14.6	696	25.1	62	2.2	2772	41.4
Moderate	171	6.2	189	6.9	645	23.4	1390	50.5	359	13.0	2754	41.1
Severe	0	0	0	0	149	12.7	576	49.1	449	38.2	1174	17.5

[1]CDC/AAP: The Centers for Disease Control and Prevention/The American Academy of Periodontology
[2]A total of 6,700 participants completed periodontal examination at Visit 4 ARIC.
[3]Five levels of BGI were the followings: BGI-DL/SB (deep lesion/severe bleeding), BGI-DL/MB (deep lesion/moderate bleeding), BGI-DL/LB (deep lesion/low bleeding), or BGI-G (gingivitis), and BGI-H (healthy).



BGI, Biofilm-Gingival Interface
BGI classified periodontal disease based on probing pocket depth (PPD) and the extent of bleeding on probing (BOP). Five levels of BGI were the followings: BGI-DL/SB (deep lesion/severe bleeding), BGI-DL/MB (deep lesion/moderate bleeding), BGI-DL/LB (deep lesion/low bleeding), or BGI-G (gingivitis), and BGI-H (healthy).

Figure C-1. Extent of probing pocket depth among Dental ARIC participants, by five levels of Biofilm-Gingival Interface classification (n = 6,700)



AL, attachment loss; BGI, Biofilm-Gingival Interface.
BGI classified periodontal disease based on probing pocket depth (PPD) and the extent of bleeding on probing (BOP). Five levels of BGI were the followings: BGI-DL/SB (deep lesion/severe bleeding), BGI-DL/MB (deep lesion/moderate bleeding), BGI-DL/LB (deep lesion/low bleeding), or BGI-G (gingivitis), and BGI-H (healthy).

Figure C-2. Extent of attachment loss of Dental ARIC participants by five levels of Biofilm-Gingival Interface classification (n = 6,700)

Table C-3. Selected characteristics of ARIC study participants at Visit 4 in relation to periodontal disease prevalence: A cross-sectional study (n = 5,966)

Characteristics (row %)	No periodontal pockets		Had periodontal pockets			*P-value*
	BOP <10%	≥ 10%	BOP < 10%	10- < 50%	≥ 50%	
Age at Visit 4 (years)						
> 65	12.7	15.2	18.0	40.1	14.0	0.0070
60 - 65	15.5	13.3	19.5	40.5	11.2	
51 - 59	14.8	15.9	17.9	39.4	12.0	
Race						
African American	24.7	19.9	10.2	24.4	20.8	<0.0001
White	12.1	13.8	20.2	43.3	10.6	
Gender						
Male	8.2	12.0	19.3	44.6	15.9	<0.0001
Female	19.5	17.3	17.7	36.0	9.5	
Study sites						
Forsyth	15.7	29.1	7.1	32.6	15.5	<0.0001
Jackson	26.6	21.2	10.4	23.6	18.1	
Minneapolis	14.3	2.5	40.6	41.1	1.6	
Washington	5.8	12.5	5.9	55.8	20.0	
Education						
Less than high school	16.1	17.7	9.5	33.5	23.2	<0.0001
High school completion	13.7	15.0	17.0	43.0	11.3	
Post-secondary education	14.6	13.8	22.3	38.8	10.4	
Income						
Refused	22.0	14.0	27.0	30.7	6.3	<0.0001
<$25,000	16.1	18.7	12.7	34.6	17.8	
$25-<$50,000	14.2	14.6	18.0	41.2	12.0	
$50,000 or more	12.9	12.7	22.0	42.7	9.7	
Cigarette use						
Current	12.2	10.7	21.0	38.3	17.8	<0.0001
Former	13.2	12.3	20.2	42.7	11.6	
Never	16.1	18.5	16.0	37.7	11.7	
Alcohol use						
Current	13.0	10.9	23.4	43.0	9.7	<0.0001
Former	15.1	16.2	15.1	38.4	15.1	
Never	17.4	24.4	8.6	33.0	16.6	
Diabetes mellitus						
Yes	10.5	18.5	13.5	38.8	18.7	<0.0001
No	14.9	14.3	19.2	40.1	11.4	
Hypertension						
Yes	15.8	15.6	14.7	39.0	14.9	<0.0001
No	13.3	14.3	21.2	40.7	10.5	
Coronary heart disease						
Yes	12.4	13.0	17.3	40.5	16.8	0.1041
No	14.5	15.0	18.5	39.9	12.1	
Stroke						
Yes	18.2	13.1	17.2	34.3	17.2	0.4135
No	14.3	14.9	18.4	40.1	12.3	
Hyperlipidemia						
Yes	13.5	12.9	18.7	42.1	12.8	0.0020
No	14.9	16.1	18.2	38.5	12.2	
Body mass index (km/m^2)						
≥ 30	13.3	15.4	14.7	41.9	14.7	<0.0001
< 30	14.9	14.6	20.2	39.0	11.3	
APOE ε4						
Yes	14.0	14.0	18.3	40.1	13.6	0.3155
No	14.5	15.2	18.5	39.9	11.9	

BGI, Biofilm-Gingival Interface

In the final analysis, African Americans from Washington and Minneapolis study sites (n = 24) were excluded, leaving an analytical sample of 5,942.

Table C-4. Selected characteristics at Visit 4 of ARIC study participants from Jackson and Forsyth County study sites in relation to periodontal disease: A longitudinal study (n = 558)

Characteristics (row %)	No periodontal pockets		Had periodontal pockets			*P-value*
	BOP <10%	≥ 10%	BOP < 10%	10- < 50%	≥ 50%	
Age at Visit 4 (years)						
> 65	17.9	33.0	6.8	28.6	13.7	0.4364
60 - 65	19.7	24.6	9.2	34.2	12.3	
51 - 59	20.3	26.6	3.1	34.4	15.6	
Race						
African American	22.6	20.8	7.5	31.4	17.7	0.0033
White	16.6	33.4	7.6	32.2	10.2	
Gender						
Male	12.7	21.3	7.2	36.6	22.2	<0.0001
Female	23.2	32.9	7.7	28.8	7.4	
Study sites						
Forsyth	16.2	33.0	7.3	31.8	11.7	0.0088
Jackson	24.0	20.0	8.0	32.0	16.0	
Education						
Less than high school	17.1	19.7	2.6	38.2	22.4	0.0429
High school completion	17.8	33.7	6.7	31.2	10.6	
Post-secondary education	20.4	26.6	9.5	30.7	12.8	
Income						
Refused	36.3	27.3	9.1	18.2	9.1	
<$25,000	20.5	26.7	4.5	29.5	18.8	0.3594
$25-<$50,000	18.7	29.0	9.3	33.2	9.8	
$50,000 or more	16.9	29.2	8.4	33.7	11.8	
Cigarette use						
Current	19.1	15.9	9.5	36.5	19.0	0.0290
Former	18.4	23.6	7.5	34.0	16.5	
Never	19.4	34.6	7.1	29.3	9.6	
Alcohol use						
Current	19.6	24.8	8.9	32.7	14.0	0.2838
Former	18.1	26.6	7.3	31.1	16.9	
Never	19.2	34.7	6.0	31.7	8.4	
Diabetes mellitus						
Yes	16.0	29.3	4.0	36.0	14.7	0.6520
No	19.5	28.2	8.1	31.3	13.0	
Hypertension						
Yes	19.2	28.5	5.1	32.3	14.9	0.4046
No	18.9	28.2	9.3	31.5	12.1	
Hyperlipidemia						
Yes	19.2	30.3	7.6	32.8	10.1	0.5866
No	18.9	27.2	7.5	31.4	15.0	
Body mass index (km/m^2)						
≥ 30	18.8	25.9	9.4	33.0	12.9	0.7778
< 30	19.1	29.4	6.7	31.4	13.4	
APOE ε4						
Yes	19.6	28.6	6.5	36.3	9.0	0.2642
No	18.7	28.2	8.0	30.0	15.1	

Number of teeth

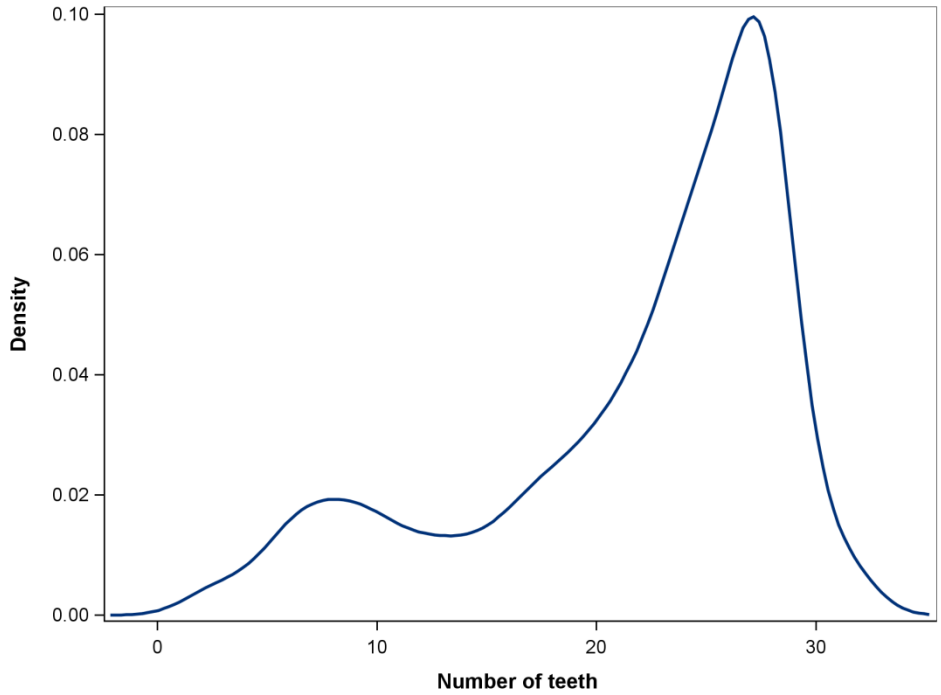
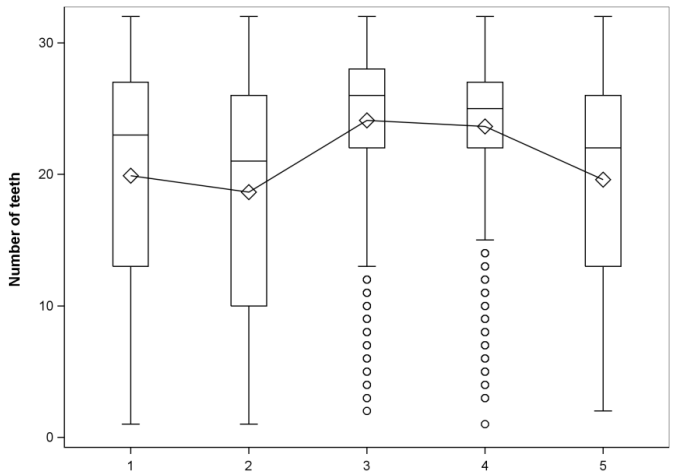
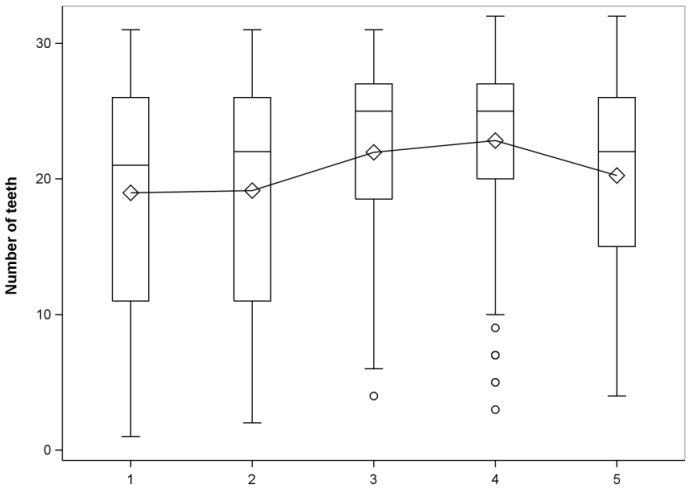


Figure C-3. Density plot for a distribution of number of teeth for Dental ARIC participants (n = 6,700)



1 = BGI-Healthy; 2= BGI-Gingivitis; 3 = BGI-Deep lesion/low bleeding; 4 = BGI-Deep lesion/moderate bleeding; 5= BGI-Deep lesion/severe bleeding

Figure C-4. Boxplots for number of teeth by five levels of Biofilm-Gingival Interface (BGI) classification for ARIC participants: A cross-sectional study (n = 5,966)[1]



1 = BGI-Healthy; 2= BGI-Gingivitis; 3 = BGI-Deep lesion/low bleeding; 4 = BGI-Deep lesion/moderate bleeding; 5= BGI-Deep lesion/severe bleeding

Figure C-5. Boxplots for number of teeth by five levels of Biofilm-Gingival Interface (BGI) classification for ARIC participants: A cohort study (n = 558)

[1]In the final analysis, African Americans from Washington and Minneapolis study sites (n = 24) were excluded, leaving an analytical sample of 5,942.

Table C-5. Selected characteristics of ARIC study participants in relation to number of teeth

Characteristics	A cross-sectional study n = 5,966[1]		A cohort study n = 558[2]	
	Col %	Mean (95% CI)	Col %	Mean (95% CI)
Age at Visit 4 (years)				
> 65	31.6	69.09 20.71, 21.34)*	41.9	20.48 (19.52, 21.43)[¥]
60 – 65	32.1	21.77 (21.45, 22.08)	46.6	20.72 (19.81, 21.63)
51 – 59	36.3	22.90 (22.60, 23.19)	11.5	21.76 (19.94, 23.59)
Race				
African American	17.6	17.59 (17.18, 18.00)*	40.5	17.28 (16.48, 18.28)*
White	82.4	22.87 (22.68, 23.06)	59.5	23.03 (22.28, 23.77)
Gender				
Male	45.8	22.10 (21.84, 22.37)[¥]	39.6	21.23 (20.25, 22.22[¥])
Female	54.2	21.81 (21.56, 22.05)	60.4	20.41 (19.62, 21.21)
Study sites				
Forsyth	26.3	22.44 (22.11, 22.78)*	64.2	22.59 (21.86, 23.32)*
Jackson	15.1	17.59 (17.15, 18.03)	35.8	17.42 (16.44, 18.40)
Minneapolis	33.3	24.13 (23.83, 24.42)	0	0
Washington	25.3	21.15 (20.81, 21.49)	0	0
Education				
Less than school	12.5	17.59 (17.10, 18.08)*	13.6	16.83 (25.19, 18.47)*
High school completion	43.3	21.50 (21.24, 21.76)	37.3	20.94 (19.95, 21.93)
Post-secondary education	44.2	23.60 (23.34, 23.87)	49.1	21.67 (20.81, 22.54)
Income				
Refused	2.1	22.82 (21.64, 23.99)*	2.0	22.00 (17.82, 26.16)*
<$25,000	24.0	18.67 (18.32, 19.02)	31.5	17.89 (16.85, 18.94)
25-<$50,000	36.2	21.71 (21.43, 22.00)	34.6	20.35 (19.35, 21.35)
$50,000 or more	37.7	24.19 (23.91, 24.47)	31.9	23.89 (22.85, 24.93)
Cigarette use				
Current	12.3	19.27 (18.77, 19.78)*	11.3	18.59 (16.75, 20.42)[§]
Former	43.9	21.82 (21.55, 22.08)	38.0	20.78 (19.78, 21.78)
Never	43.8	22.82 (22.55, 23.09)	50.7	21.19 (20.32, 22.05)
Alcohol use				
Current	55.0	23.12 (22.88, 23.36)*	38.4	22.97 (22.00, 23.94)*
Former	26.3	20.69 (20.34, 21.03)	31.7	19.65 (18.58, 20.72)
Never	18.7	20.25 (19.84, 20.66)	29.9	19.04 (17.93, 20.14)
Diabetes mellitus				
Yes	13.3	19.86 (19.27, 20.35)*	13.4	18.69 (17.01, 20.37)[§]
No	86.7	22.26 (22.07, 22.45)	86.6	21.06 (20.40, 21.72)
Hypertension				
Yes	42.5	20.94 (20.66, 21.21)*	42.1	19.44 (18.49, 20.38)*
No	57.5	22.69 (22.45, 22.92)	57.9	21.69 (20.88, 22.49)
Hyperlipidemia				
Yes	39.0	21.77 (21.48, 22.06)[¥]	35.5	20.64 (19.60, 21.68)[¥]
No	61.0	22.05 (21.82, 22.28)	64.5	20.79 (20.02, 21.57)
Body mass index (km/m^2)				
≥ 30	32.6	22.42 (22.20, 22.64)*	30.5	18.15 (17.05, 19.24)*
< 30	67.4	20.96 (20.64, 21.27)	69.5	21.88 (21.15, 22.60)
APOE ε4				
Yes	30.1	21.59 (21.26, 21.92)[§]	30.1	19.59 (18.47, 20.72)[§]
No	69.9	22.09 (21.88, 22.31)	69.9	

[1]Participants were from all four study sites. In the final analysis, African Americans from Washington and Minneapolis study sites (n = 24) were excluded, leaving an analytical sample of 5,942.
[2]Paticipants were from two study sites (Jackson MS and Forsyth County NC).
*$p < 0.0001$
[§]$p < 0.05$
[¥]$p > 0.05$

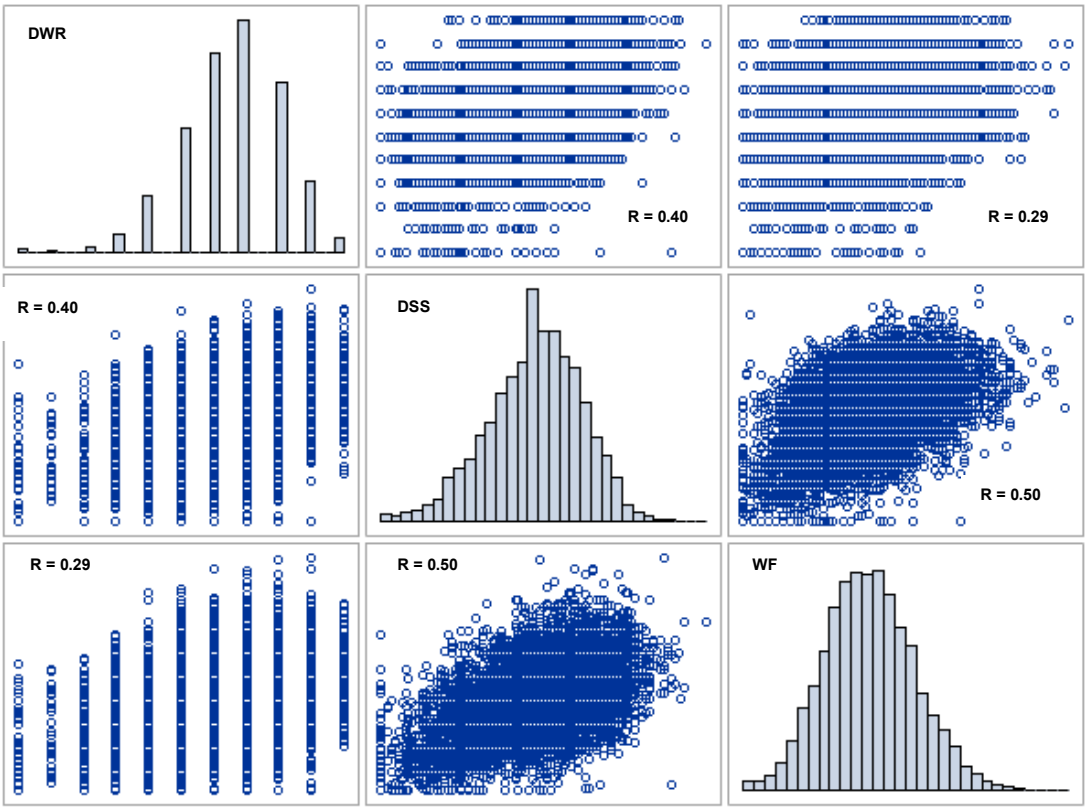
Appendix D: Cognitive function assessment in ARIC study

Over a 14-year follow-up in the ARIC study, cognitive scores of ARIC participants slightly decreased for both whites and African Americans (Table 7-10).The latter had lower scores at each time point, consistent with racial difference in education attainment. The present study used cognitive scores measured at Visit 4 as baseline scores.

Table D-1. Means, standard deviations, and median scores of three cognitive tests for African American and white participants at each ARIC visit

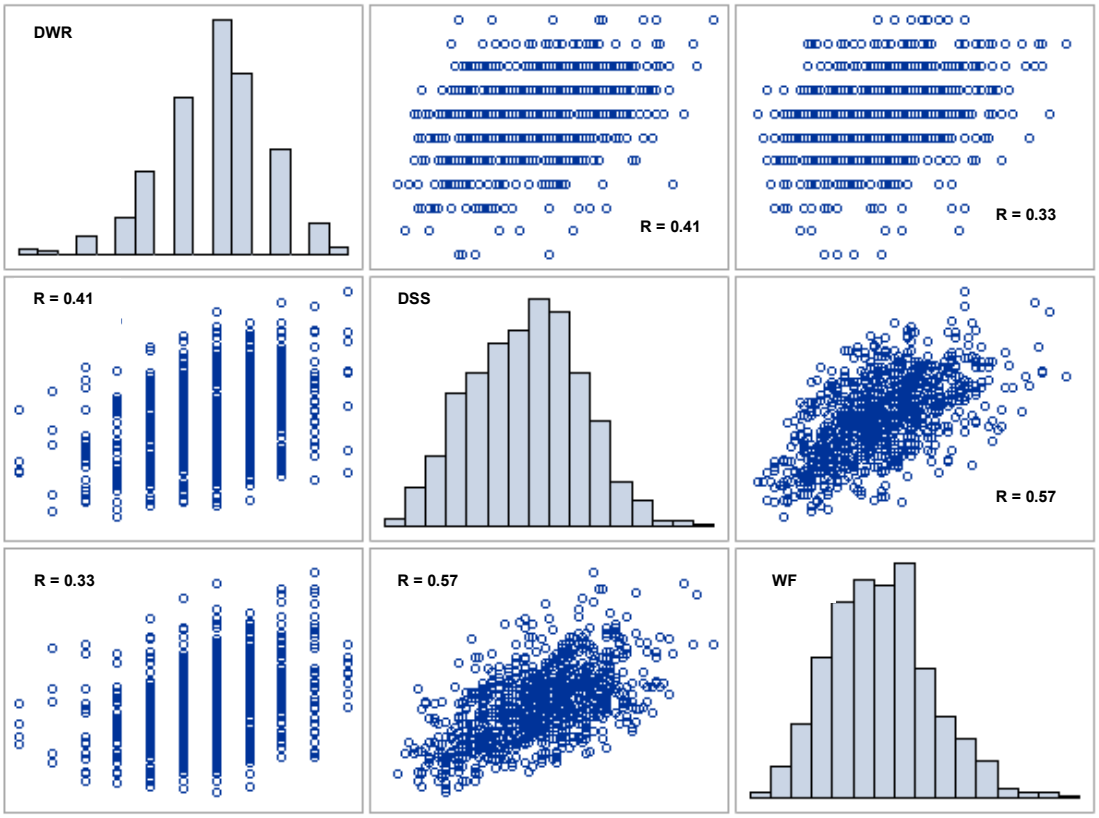
Visit	African American			White		
	n	Mean ± SD	Median	n	Mean ± SD	Median
1990-1992 (Visit 2)						
Delayed word recall	3,389	6.1 ± 1.7	6	10,626	6.8 ± 1.4	7
Digit symbol substitution	3,352	31.1 ± 13.4	30	10,614	48.9 ± 11.6	49
Word fluency	3,375	27.7 ± 13.1	27	10,620	34.9 ± 11.8	34
1993-1995 (Visit 3)						
Delayed word recall	995	6.0 ± 1.8	6	1,052	6.7 ± 1.5	7
Digit symbol substitution	972	28.8 ± 13.0	28	1,048	46.3 ± 11.3	46
Word fluency	985	27.6 ± 13.3	26	1,053	33.8 ± 11.6	33
1996-1998 (Visit 4)						
Delayed word recall	2,324	6.0 ± 1.7	6	8,846	6.7 ± 1.5	7
Digit symbol substitution	2,293	30.6 ± 13.4	30	8,825	46.8 ± 11.3	47
Word fluency	2,309	27.9 ± 13.3	27	8,839	34.8 ± 12.0	34
2004-2006 (Brain MRI)						
Delayed word recall	541	5.4 ± 1.8	6	536	6.3 ± 1.6	6
Digit symbol substitution	529	28.8 ± 12.0	27	533	43.7 ± 10.5	43
Word fluency	538	26.9 ± 12.4	25	537	34.9 ± 11.4	34

n, total number of participants who completed each cognitive function tests; SD, Standard deviation



DWR, Delayed Word Recall; DSS, Digit Symbol Substitution; WF, Word Fluency test;
R = Rho (Pearson correlation coefficient)

Figure D-1. Pearson correlation coefficients among three cognitive scores at 1996-1998 for ARIC participants (n = 11,097)



DWR, Delayed word recall; DSS, Digit symbol substitution; WF, Word fluency test;
R = Rho (Pearson correlation coefficient)

Figure D-2. Pearson correlation coefficients among three cognitive function scores at 2004-2006 for ARIC participants (n = 1,101)

Table D-2. Cognitive scores at Visit 4 in relation to selected study characteristics (n = 9,909)[1]

Characteristics	Col %	Mean (95% CI)		
		Delayed word recall	Digit symbol substitution	Word fluency
Age at Visit 4 (years)				
> 65	34.3	6.17 (6.12, 6.22)*	39.70 (39.27, 40.14)*	32.44 (32.02, 32.86)*
60-65	31.8	6.66 (6.60, 6.71)	44.48 (44.03, 44.93)	33.50 (33.06, 33.93)
51-59	33.9	6.90 (6.85, 6.95)	47.21 (46.77, 47.65)	34.94 (34.52, 35.36)
Gender				
Male	44.3	6.20 (6.15, 6.24)*	41.45 (41.06, 41.84)*	32.57 (32.20, 32.94)*
Female	55.7	6.87 (6.83, 6.91)	45.60 (45.26, 45.95)	34.46 (34.13, 34.79)
Race				
African American	20.5	6.06 (5.99, 6.12)*	31.21 (30.70, 31.72)*	28.57 (28.04, 29.10)*
White	79.5	6.70 (6.67, 6.74)	47.00 (46.74, 47.26)	34.92 (34.66, 35.20)
Study sites				
Forsyth	25.2	6.77 (6.71, 6.83)*	45.51 (45.05, 45.96)*	33.52 (33.04, 34.00)*
Jackson	17.8	6.02 (5.95, 6.10)	30.32 (29.77, 30.86)	28.28 (27.71, 28.85)
Minneapolis	29.2	6.74 (6.68, 6.79)	49.06 (48.64, 49.48)	36.60 (36.15, 37.04)
Washington	27.8	6.56 (6.50, 6.62)	45.21 (44.77, 45.64)	34.01 (33.55, 34.46)
Education				
Less than high school	17.7	5.91 (5.84, 5.98)*	31.81 (31.25, 32.37)*	24.39 (23.86, 24.92)*
High school completion	42.6	6.62 (6.57, 6.66)	44.33 (43.97, 44.69)	32.58 (32.23, 32.92)
Post-secondary education	39.7	6.82 (6.77, 6.86)	48.50 (48.12, 48.87)	38.87 (38.52, 39.23)
Income				
Refused	2.2	6.27 (6.06, 6.47)*	40.63 (39.01, 42.24)*	31.33 (29.74, 32.92)*
<$25,000	30.2	6.18 (6.12, 6.23)	36.30 (35.87, 36.74)	28.72 (28.29, 29.15)
$25-<$50,000	35.0	6.60 (6.55, 6.65)	44.70 (44.30, 45.11)	34.16 (33.76, 34.56)
$50,000 or more	32.6	6.92 (6.87, 6.97)	49.88 (49.46, 50.30)	37.74 (37.33, 38.15)
Cigarette use				
Current	14.4	6.45 (6.36, 6.53)*	40.46 (39.77, 41.14)*	31.82 (31.18, 32.47)*
Former	43.9	6.50 (6.45, 6.55)	44.04 (43.64, 44.43)	34.12 (33.75, 34.49)
Never	41.6	6.69 (6.64, 6.74)	44.63 (44.22, 45.03)	33.73 (33.35, 34.11)
Alcohol use				
Current	50.3	6.71 (6.67, 6.76)*	47.31 (46.95, 47.66)*	36.12 (35.78, 36.46)*
Former	29.5	6.42 (6.36, 6.48)	40.74 (40.28, 41.21)	31.71 (31.27, 32.16)
Never	20.1	6.44 (6.37, 6.50)	39.34 (38.79, 39.91)	30.18 (29.64, 30.71)
Diabetes mellitus				
Yes	15.8	6.19 (6.11, 6.27)*	38.51 (37.86, 39.16)*	30.41 (29.80, 31.03)*
No	84.2	6.64 (6.61, 6.68)	44.75 (44.47, 45.03)	34.22 (33.96, 34.49)
Hypertension				
Yes	46.6	6.40 (6.36, 6.45)*	40.92 (40.55, 41.30)*	32.26 (31.89, 32.61)*
No	53.4	6.72 (6.68, 6.76)	46.25 (45.90, 46.60)	34.82 (34.49, 35.16)
Coronary heart disease				
Yes	8.4	6.10 (6.00, 6.21)*	39.48 (38.57, 40.38)*	31.17 (30.32, 32.02)*
No	91.6	6.61 (6.58, 6.65)	44.16 (43.88, 44.43)	33.85 (33.59, 34.10)
Stroke				
Yes	2.1	5.56 (5.34, 5.77)*	33.18 (31.38, 34.98)*	28.65 (26.96, 30.35)*
No	97.9	6.59 (6.56, 6.62)	43.99 (43.73, 44.26)	33.73 (33.48, 33.98)
Hyperlipidemia				
Yes	40.0	6.54 (6.49, 6.59)¥	43.55 (43.14, 43.97)¥	33.30 (32.91, 33.69)§
No	60.0	6.59 (6.55, 6.63)	43.91 (43.57, 44.25)	33.84 (33.52, 34.16)

Body mass index (kg/m^2)				
≥ 30	34.4	6.52 (6.46, 6.57)[§]	42.09 (41.64, 42.53)*	32.55 (32.13, 32.97)*
< 30	65.6	6.60 (6.56, 6.64)	44.64 (44.32, 45.97)	34.19 (33.89, 34.49)
APOE ε4				
Yes	29.9	6.45 (6.39, 6.51)*	42.16 (41.69, 42.64)*	33.23 (32.78, 33.68)[§]
No	70.1	6.62 (6.58, 6.66)	44.45 (44.14, 44.76)	33.79 (33.50, 34.08)
Oral health conditions				
Edentulous				
Yes	13.4	6.07 (5.99, 6.16)*	35.63 (34.93, 36.32)*	27.68 (27.02, 28.34)*
No	86.6	6.65 (6.61, 6.68)	45.02 (44.75, 45.29)	34.28 (34.28, 34.80)
Number of teeth[2]				
1-24	50.5	6.48 (6.42, 6.53)*	42.34 (41.91, 42.77)*	32.71 (32.27, 33.14)*
≥ 25	49.5	6.86 (6.81, 6.92)	49.00 (48.57, 49.44)	36.99 (36.55, 37.42)
Periodontal disease[2]				
Had periodontal pockets				
BOP ≥ 50%	12.4	6.39 (6.28, 6.50)*	41.20 (40.30, 42.07)*	31.80 (30.92, 32.68)*
BOP 10- <50%	40.0	6.68 (6.61, 6.74)	46.88 (46.38, 47.37)	35.32 (34.83, 35.81)
BOP < 10%	18.4	6.72 (6.63, 6.81)	48.08 (47.35, 48.80)	37.40 (36.67, 38.12)
No periodontal pockets				
BOP ≥ 10%	14.8	6.79 (6.68, 6.89)	43.72 (42.91, 44.53)	33.06 (32.26, 33.87)
BOP < 10%	14.4	6.69 (6.59, 6.80)	44.92 (44.09, 45.74)	34.58 (33.77, 35.40)

n, total number of study group; BGI, Biofilm-Gingival Interface

[1]Participants were from all four study sites. In the final analysis, African Americans from Washington and Minneapolis study sites (n =35) were excluded, leaving an analytical sample of 9,874.

[2]A subset of dentate participants received periodontal examination (n = 5,966). In the final analysis, African Americans from Washington and Minneapolis study sites (n =24) were excluded, leaving an analytical sample of 5,942.

BGI classified periodontal disease based on probing pocket depth (PPD) and the extent of bleeding on probing (BOP).

*$p < 0.0001$

[§] $p < 0.05$

[¥] $p > 0.05$

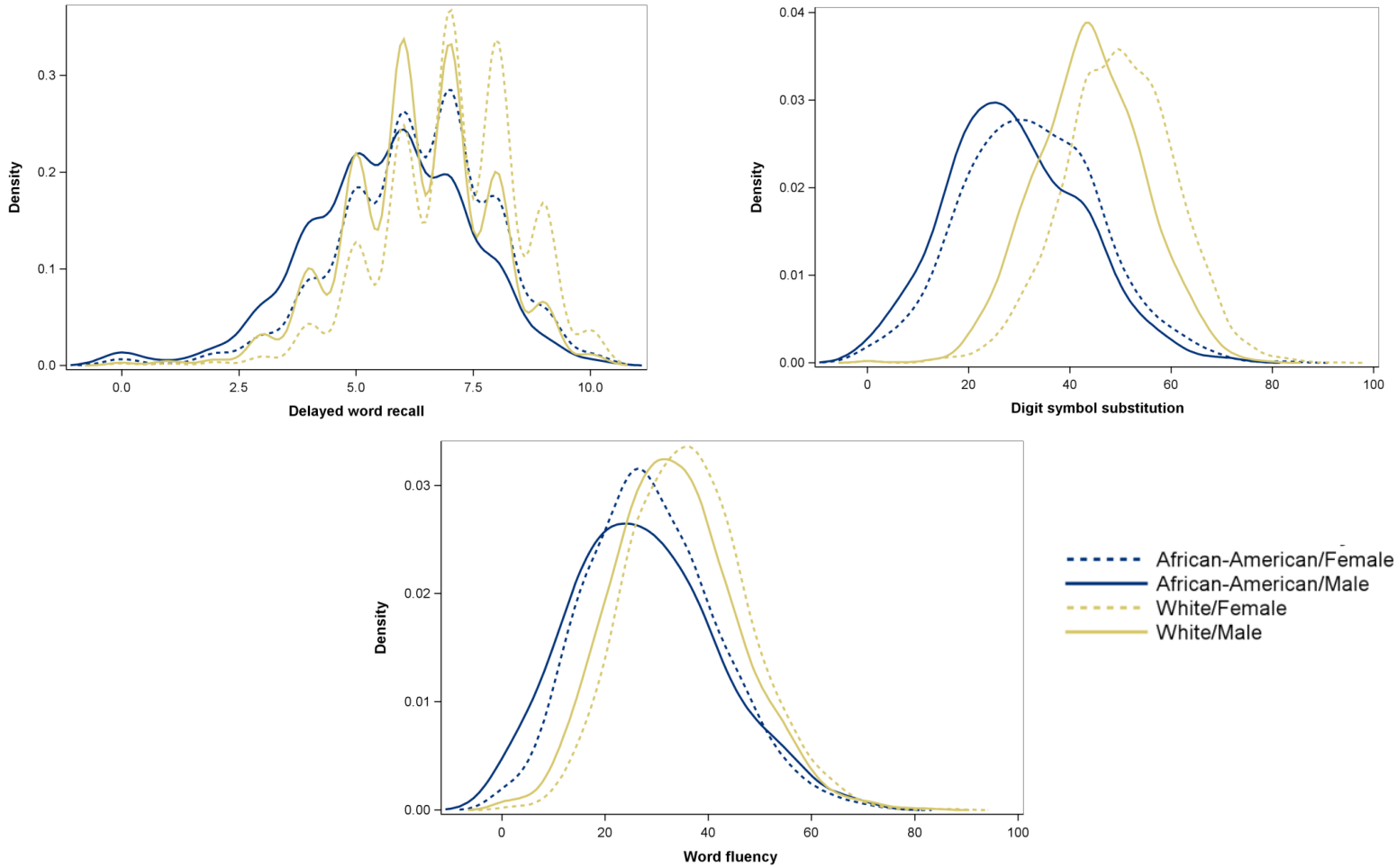


Figure D-3. Race- and gender-specific density plots for three cognitive scores measured at Visit 4: A cross-sectional study (n = 9,909)[1]

[1]Participants were from all four study sites. In the final analysis, African Americans from Washington and Minneapolis study sites (n =35) were excluded, leaving an analytical sample of 9,874.

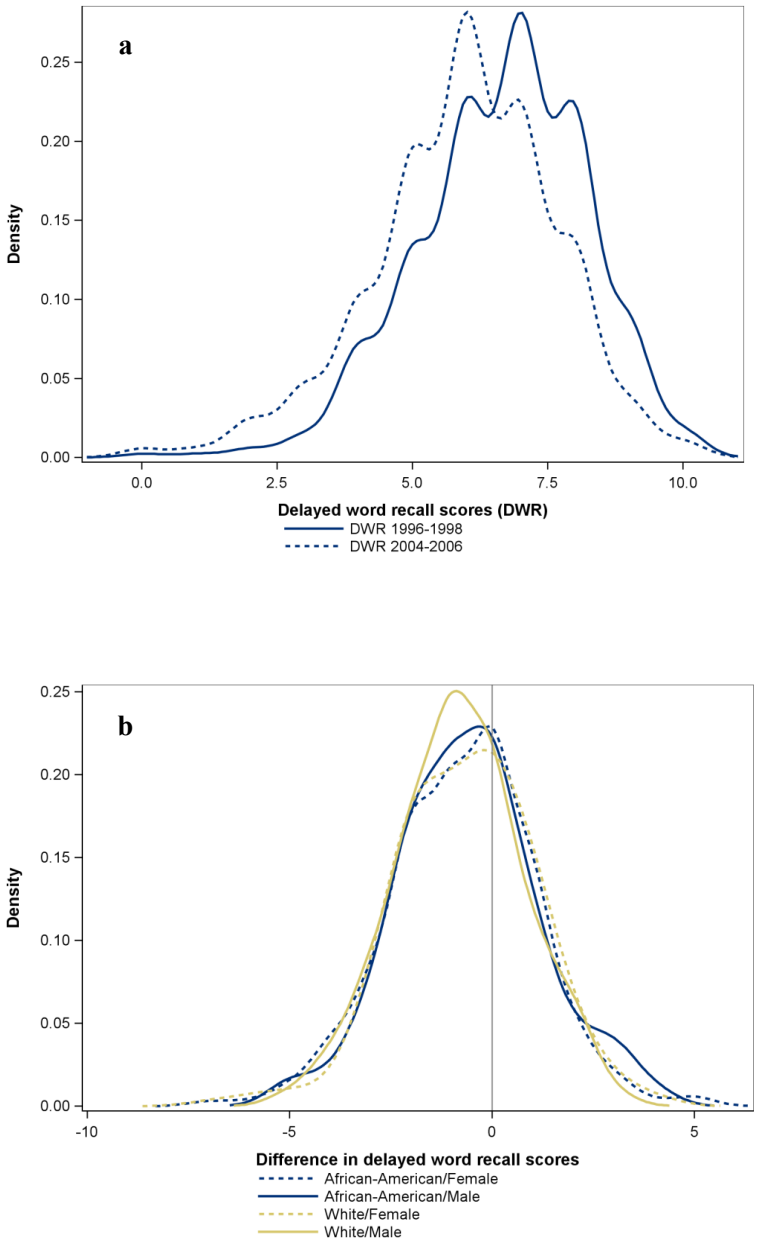


Figure D-4**.** Density plots for a) Delayed Word Recall scores at 1996-1998 and 2004-2006; b) race- and gender-specific changes in Delayed Word Recall scores: A cohort study (n = 911)

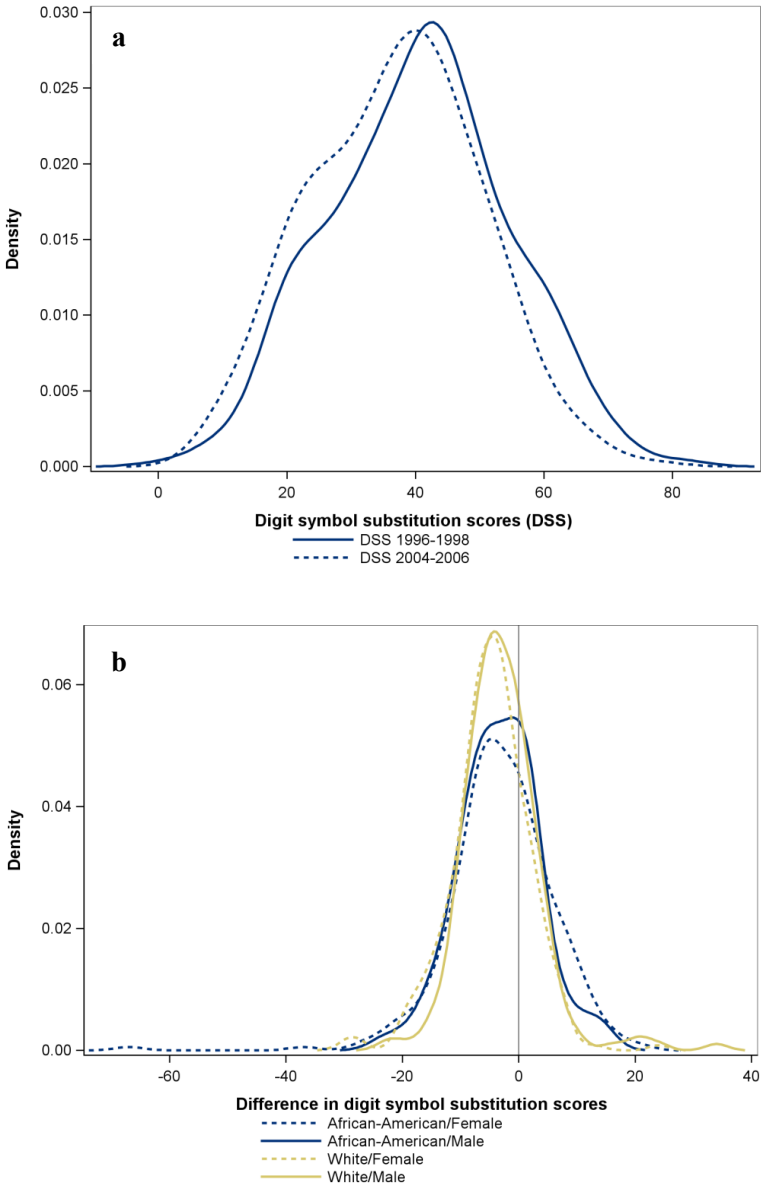


Figure D-5**.** Density plots for a) Digit Symbol Substitution scores at 1996-1998 and 2004-2006; b) race- and gender-specific changes in Digit Symbol Substitution scores: A cohort study (n = 911)

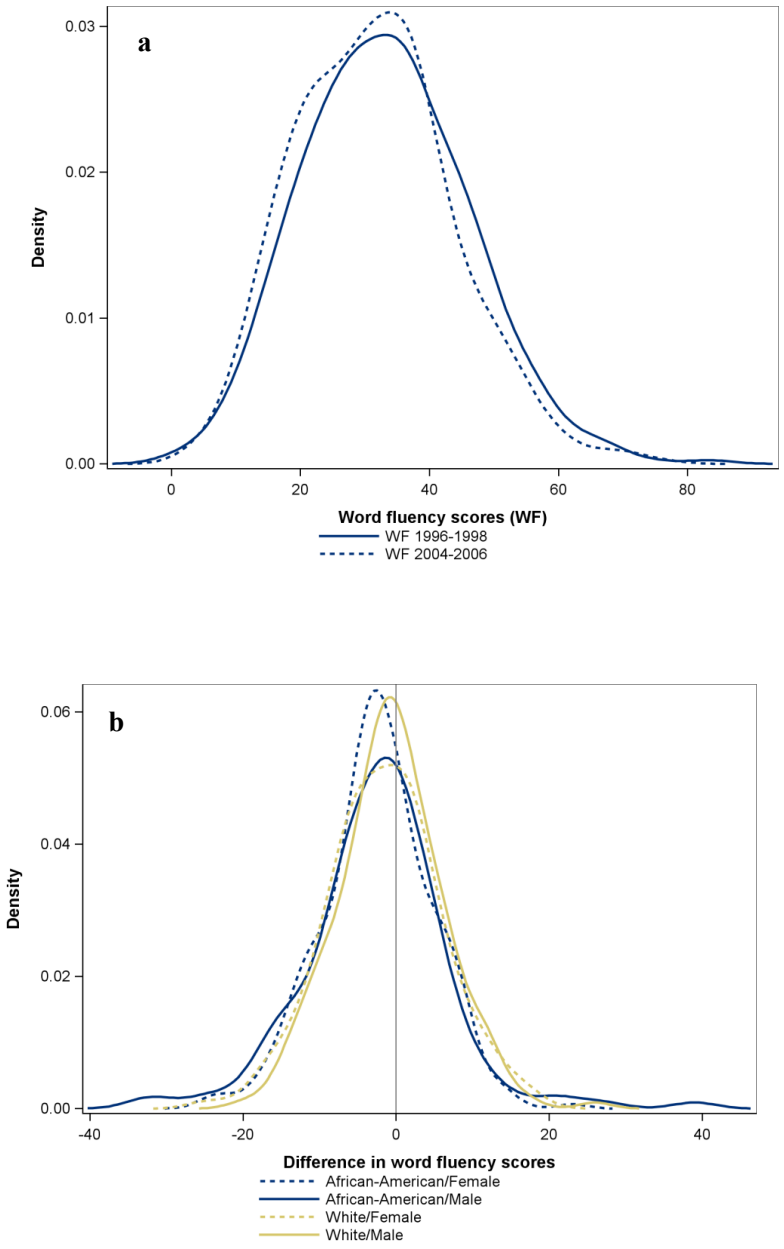


Figure D-6. Density plots for a) Word Fluency scores at 1996-1998 and 2004-2006; b) race- and gender-specific changes in Word Fluency scores: A cohort study (n = 911)

Table E-1. Characteristics of ARIC cohort members who were included in or excluded from cross-sectional and cohort studies

Characteristics	Aim 1: A Cross-sectional study				Aim 2: A Longitudinal study							
	Excluding		Study sample		Minneapolis &Washington		Forsyth & Jackson				ARIC Visit 4	
							Excluding		Study sample			
	n = 1,568		n = 9,874		n = 6,319		n = 4,212		n = 911		n =11,442[1]	
Age at Visit 4 (mean, SD)	62.9	5.7	62.8	5.7	63.0	5.5	62.1	6.0	64.7	4.3	62.8	5.7
Race (n, %)												
African American	561	35.8	1,994	20.2	0	0.0	2,113	50.2	442	48.5	2,555	22.3
White	1,007	64.2	7,880	79.8	6,319	100.0	2,099	49.8	469	51.5	8,887	77.7
Gender (n, %)												
Female	874	55.7	5510	55.8	3,369	53.3	2,456	58.3	559	61.4	6,384	55.8
Male	694	44.3	4364	44.2	2,950	46.7	1,756	41.7	352	38.6	5,058	44.2
Study site (n, %)												
Forsyth	321	20.5	2,503	25.3	0	0	2,306	54.7	518	56.9	2,824	24.7
Jackson	541	34.5	1,758	17.8	0	0	1,906	45.3	393	43.1	2,299	20.1
Minneapolis	318	20.3	2,878	29.2	3,196	50.58	0	0	0	0	3,196	27.9
Washington	388	24.7	2,735	27.7	3,123	49.42	0	0	0	0	3,123	27.3
Education (n, %)												
Missing	16	1.0	0	0	5	0.1	11	0.3	0	0	16	0.1
Less than high school	468	29.8	1,742	17.6	959	15.2	1,069	25.4	182	20.0	2,210	19.3
High school completion	603	38.5	4,213	42.7	2,965	46.9	1,534	36.4	317	34.8	4,816	42.1
Post-secondary education	481	30.7	3,919	39.7	2,390	37.8	1,598	37.9	412	45.2	4,400	38.5
Income (n, %)												
Missing	310	19.8	0	0	68	1.1	242	5.7	0	0	310	2.7
Refused	39	2.5	217	2.2	114	1.8	120	2.8	22	2.4	256	2.2
<$25,000	490	31.3	2,978	30.2	1,544	24.4	1,573	37.3	351	38.5	3,468	30.3
$25-<$50,000	405	25.8	3,460	35.0	2,410	38.1	1,169	27.8	286	31.4	3,865	33.8
$50,000 or more	324	20.7	3,219	32.6	2,183	34.5	1,108	26.3	252	27.7	3,543	31.0

Cigarette use (n, %)												
Missing	112	7.1	0	0	5	0.1	107	2.5	0	0	112	1.0
Current	258	16.5	1,426	14.4	807	12.8	758	18.0	119	13.1	1,684	14.7
Former	606	38.6	4,338	43.9	2,963	46.9	1,627	38.6	354	38.9	4,944	43.2
Never	592	37.8	4,110	41.6	2,544	40.3	1,720	40.8	438	48.1	4,702	41.1
Alcohol use (n, %)												
Missing	110	7.0	0	0	5	0.1	105	2.5	0	0	110	1.0
Current	606	38.6	4,973	50.4	3,836	60.7	1,417	33.6	326	35.8	5,579	48.8
Former	482	30.7	2,911	29.5	1,642	26.0	1,441	34.2	310	34.0	3,393	29.7
Never	370	23.6	1,990	20.2	836	13.2	1,249	29.7	275	30.2	2,360	20.6
Diabetes (n, %)												
Missing	143	9.1	0	0	19	0.3	124	2.9	0	0	143	1.3
No	1,074	68.5	8,317	84.2	5,400	85.5	3,232	76.7	759	83.3	9,391	82.1
Yes	351	22.4	1,557	15.8	900	14.2	856	20.3	152	16.7	1,908	16.7
Hypertension (n, %)												
Missing	56	3.6	0	0	24	0.4	32	0.8	0	0	56	0.5
No	671	42.8	5,270	53.4	3,535	55.9	1,936	46.0	470	51.6	5,941	51.9
Yes	841	53.6	4,604	46.6	2,760	43.7	2,244	53.3	441	48.4	5,445	47.6
Coronary heart disease (n, %)												
Missing	219	14.0	0	0	128	2.0	75	1.8	16	1.8	219	1.9
No	1,200	76.5	9,047	91.6	5,620	88.9	3,774	89.6	853	93.6	1,0247	89.6
Yes	149	9.5	827	8.4	571	9.0	363	8.6	42	4.6	976	8.5
Stroke (n, %)												
Missing	23	1.5	0	0	13	0.2	9	0.2	1	0.1	23	0.2
No	1,483	94.6	9,667	97.9	6,182	97.8	4,075	96.7	893	98.0	11,150	97.4
Yes	62	4.0	207	2.1	124	2.0	128	3.0	17	1.9	269	2.4
Body mass index (n, %)												
Missing	38	2.4	0	0	13	0.2	25	0.6	0	0	38	0.3
< 30 kg/m^2	928	59.2	6,482	65.6	4,111	65.1	2,675	63.5	624	68.5	7,410	64.8
≥ 30 kg/m^2	602	38.4	3,392	34.4	2,195	34.7	1,512	35.9	287	31.5	3,994	34.9
Hyperlipidemia (n, %)												
Missing	227	14.5	0	0	84	1.3	143	3.4	0	0	227	2.0
No	798	50.9	5,925	60.0	3,582	56.7	2,547	60.5	594	65.2	6,723	58.8
Yes	543	34.6	3,949	40.0	2,653	42.0	1,522	36.1	317	34.8	4,492	39.3

APOE ε4 (n, %)												
Missing	439	28.0	0	0	253	4.0	186	4.4	0	0	439	3.8
No	745	47.5	6,924	70.1	4,370	69.2	2,667	63.3	632	69.4	7,669	67.0
Yes	384	24.5	2,950	29.9	1,696	26.8	1,359	32.3	279	30.6	3,334	29.1
Oral health conditions												
Dental status (n, %)												
Missing	126	8.0	0	0	72	1.1	54	1.3	0	0	126	1.1
Edentulous	270	17.2	1,320	13.4	766	12.1	698	16.6	126	13.8	1,590	13.9
Dentate	1,172	74.7	8,554	86.6	5,481	86.7	3,460	82.1	785	86.2	9,726	85.0
No. of teeth, (mean, SD)	20.3	7.4	22	7.1	22.7	6.7	20.4	7.5	20.7	7.5	21.8	7.1
N/A	835		3,931		2,491		1,992		353		4,766	
BGI index (n, %)												
N/A	835	53.3	3,932	39.8	2,491	39.4	1,923	45.7	353	38.7	4,767	41.7
Had periodontal pockets												
BOP ≥ 50%	130	8.3	733	7.4	370	5.9	419	9.9	74	8.1	863	7.5
BOP 10- <50%	278	17.7	2,374	24.0	1,824	28.9	650	15.4	178	19.5	2,652	23.2
BOP < 10%	99	6.3	1,097	11.1	957	15.1	197	4.7	42	4.6	1,196	10.5
No periodontal pockets												
BOP ≥ 10%	126	8.0	884	9.0	277	4.4	575	13.7	158	17.3	1,010	8.8
BOP < 10%	100	6.4	854	8.6	400	6.3	448	10.6	106	11.6	954	8.3
CDC/AAP (n, %)												
N/A	835	53.3	3,931	39.8	2,491	39.4	1,922	45.6	353	38.7	4,766	41.7
Healthy/Mild	293	18.7	2,475	25.1	1,357	21.5	1,133	26.9	278	30.5	2,768	24.2
Moderate	309	19.7	2,434	24.7	1,730	27.4	808	19.2	205	22.5	2,743	24.0
Severe	131	8.4	1,034	10.5	741	11.7	349	8.3	75	8.2	1,165	10.2
Cognitive function												
DWR (mean, SD)	6.4	1.7	6.6	1.6	6.6	1.5	6.4	1.7	6.7	1.6	6.5	1.6
Missing	310		0		22		228		0		310	
DSS (mean, SD)	41.2	14.5	43.8	13.3	47.0	11.2	38.5	14.9	40.5	14.0	43.5	13.5
Missing	362		0		37		325		0		362	
WF (mean, SD)	31.2	12.8	33.6	12.5	35.2	11.8	30.5	13.1	33.3	12.9	33.4	12.6
Missing	332		0		28		304		0		332	

SD, standard deviation; CDC/AAP, The Centers for Disease Control and Prevention/The American Academy of Periodontology; BGI, Biofilm-Gingival classification; DWR, Delayed Word Recall; DSS, Digit Symbol Substitution; WF, Word Fluency; BGI, Biofilm-Gingival Interface

BGI classified periodontal disease based on probing pocket depth (PPD) and the extent of bleeding on probing (BOP).

[1]African Americans from Washington and Minneapolis sties (n = 38) were excluded.

Table E-2. Selected characteristics of ARIC study participants by study sites: A cross-sectional study

Characteristics	Forsyth County		Jackson		Minneapolis		Washington County	
	n = 2,503		n = 1,758		n = 2,878		n = 2,735	
Age at Visit 4, (mean, SD)	63.3	5.9	61.4	5.6	62.7	5.5	63.4	5.5
African American (n, %)	236	9.4	1,758	100.0	0	0	0	0
Male (n, %)	1,138	45.5	609	34.6	1,359	47.2	1,258	46.0
Education								
Less than high school	324	12.9	595	33.8	163	5.7	660	24.1
High school completion	1,060	42.3	515	29.3	1,318	45.8	1,320	48.3
Post-secondary education	1,119	44.7	648	36.9	1,397	48.5	755	27.6
Income (n, %)								
Refused	68	2.7	49	2.8	60	2.1	40	1.5
< $25,000	573	22.9	1042	59.3	448	15.6	915	33.5
$25-<$50,000	891	35.6	405	23.0	1,127	39.2	1,037	37.9
$50,000 or more	971	38.8	262	14.9	1,243	43.2	743	27.2
Cigarette use (n, %)								
Current	437	17.5	280	15.9	368	12.8	341	12.5
Former	1,072	42.8	627	35.7	1,443	50.1	1,196	43.7
Never	994	39.7	851	48.4	1,067	37.1	1,198	43.8
Alcohol use (n, %)								
Current	1,056	42.2	457	26.0	2,134	74.1	1,326	48.5
Former	809	32.3	674	38.3	604	21.0	824	30.1
Never	638	25.5	627	35.7	140	4.9	585	21.4
Diabetes mellitus (n, %)	349	13.9	449	25.5	305	10.6	454	16.6
Hypertension (n, %)	1,000	40.0	1,179	67.1	1,142	39.7	1,283	46.9
Coronary heart disease (n, %)	222	8.9	105	6.0	210	7.3	290	10.6
Stroke (n, %)	50	2.0	52	3.0	49	1.7	56	2.0
Hyperlipidemia (n, %)	921	36.8	642	36.5	1,170	40.7	1,216	44.5
Body mass index ≥ 30 kg /cm^2	609	24.3	854	48.6	921	32.0	1,008	36.9
APOE ε 4 (n, %)	676	27.0	713	40.6	825	28.7	736	26.9
Edentulous (n, %)	297	11.9	350	19.9	156	5.4	517	18.9
Number of teeth[1] (mean, SD)	22.4	6.8	17.6	7.6	24.1	5.7	21.2	7.3
Periodontal disease[1] (n, %)								
Had periodontal pockets								
BOP ≥ 50%	243	15.5	163	18.1	30	1.5	297	19.8
BOP 10- < 50%	511	32.6	213	23.6	811	41.1	839	56.0
BOP < 10%	112	7.1	94	10.4	802	40.6	89	5.9
No periodontal pockets								
BOP ≥ 10%	456	29.1	191	21.2	49	2.5	188	12.5
BOP < 10%	246	15.7	240	26.6	282	14.3	86	5.7
Delayed Word Recall (mean, SD)	6.8	1.6	6	1.7	6.7	1.5	6.6	1.5
Digit Symbol Substitution (mean , SD)	45.5	11.9	30.3	13.2	49.1	10.7	45.3	11.3
Word fluency (mean, SD)	33.5	12.4	28.3	13.2	36.6	11.3	34.1	12.3

SD, standard deviation; BGI, Biofilm-Gingival Interface

[1]A subset of dentate participants received dental examination (n = 5,942)

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